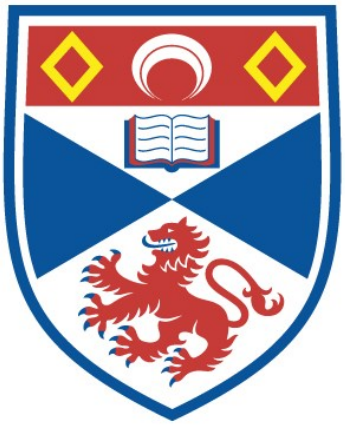
THE CHEMISTRY OF GLUCOSONE

John Anthony Fewster

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



1953

THE CHEMISTRY OF

GLUCOSONE

BY

JOHN A. FEWSTER, B.Sc.

A THESIS PRESENTED TO THE

UNIVERSITY OF ST. ANDREWS

FOR THE

DEGREE OF DOCTOR OF PHILOSOPHY

1953

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

MS 1,486

DECLARATION.

I hereby declare that the following thesis is based on work carried out by me, that the thesis is my own composition, and that no part of it has been presented previously for a Higher Degree.

The research was carried out in the Department of Physiology and Biochemistry in the United College of St. Salvator and St. Leonard, St. Andrews, under the direction of Alexander Hynd, M.A., D.Sc., and, later, Stephen Bayne, B.Sc., M.B., Ch.B.

J. A. Fewster.

CERTIFICATES.

I hereby certify that JOHN ANTHONY FEWSTER has spent three terms engaged in research work under my direction.

A. Hynd.

I hereby certify that JOHN ANTHONY FEWSTER has spent seven terms engaged in research work under my direction, and that he has fulfilled the conditions of Ordinance 16 (St. Andrews), and is qualified to submit the accompanying thesis for the Degree of Doctor of Philosophy.

Stephen Bayne.

CAREER.

I first matriculated in the University of St. Andrews in October, 1945, and followed a course of study leading to graduation in Science (Biochemistry and Chemistry) in June, 1948. Thereafter I was awarded First Class Honours in Biochemistry in June, 1949.

I was accepted as a research student in September, 1949. From October, 1949, until September, 1951, I held a Carnegie Trust for the Universities of Scotland Research Scholarship. In October, 1951, I was appointed Assistant in Biochemistry in the Department of Physiology and Biochemistry, United College of St. Salvator and St. Leonard, St. Andrews.

J. A. Fewster.

THE CHEMISTRY OF GLUCOSONE.

CONTENTS.

PREFACE.

Although the osones were first prepared by Emil Fischer sixty-five years ago and, in the intervening period, investigation of these compounds has not been neglected, there exists no modern comprehensive review of their chemistry and biochemistry. In consequence, Part I of this thesis is a full and critical survey of work published on the osones up to June, 1953.

Part II, a discussion of the methods and results of the author's own research on the subject, is presented under the same arrangement of sub-titles as that used in Part I. The object of this research was to investigate the preparation and properties of the osones, in particular D-glucosone, and their derivatives by the application of both modern and classical methods of carbohydrate chemistry, with a view to establishing the structural features of these compounds. An attempt has been made to correlate the author's own results with those of other workers and, at the same time, to develop lines of attack which have hitherto received no attention.

Part III is a detailed report of the author's experimental work.

The material presented stands as a contribution to carbohydrate chemistry. That further investigation of the osones is justified may be realised from consideration of the following facts: no completely satisfactory methods for the preparation, characterisation, and estimation of a pure osone have been evolved; no osone has been crystallised and no preparation of a crystalline derivative from which the osone may be readily regenerated has been described; there is little definitive knowledge of osone structure. In addition, the elucidation of the precise chemical structure of D-glucosone and its behaviour in solution is essential for the complete understanding of the biological significance of the compound.

The author is greatly indebted to (the late) Principal Sir James Colquhoun Irvine, K.B.E., F.R.S., for kindly advice and every encouragement, to Professor A. E. Ritchie for advice on the presentation of the material, to Dr. A. Hynd and Dr. S. Bayne for supervision, to Professor E. L. Hirst, F.R.S., Dr. D. J. Bell and Dr. J. W. H. Oldham for helpful discussion and criticism, to Professor H. J. Rose, F.B.A., and Dr. A. Cysar for assistance in the translation of original papers, to Mr. I. L. S. Mitchell for acetone determinations, and to Mr. E. Carstairs for assistance with the photographic work; also to the Carnegie Trust for the Universities of Scotland for a Research Scholarship.

J. A. F.

The United College,
St. Andrews.

August, 1953.

PART I: A REVIEW OF THE PRESENT KNOWLEDGE REGARDING THE OSONES.

CONTENTS.

1. THE FORMATION & PREPARATION OF OSONES.

1.1. Introduction.

Fischer's synthesis in 1884 of the phenylosazones of the reducing sugars, a sequel to his synthesis of phenylhydrazine, initiated his celebrated carbohydrate researches. In 1888 he published the first recorded account of the preparation of an osone, by the decomposition of D-glucose phenylosazone.

Fischer (1888) first called the compound "oxyglucose", but later (1889) renamed it glucosone since the former name suggested the presence of an extra hydroxyl group, whereas the change from glucose, as he visualised it, involved dehydrogenation of the secondary alcoholic group adjacent to the reducing portion of the molecule to a ketone group. The general term of osones was applied to the compounds obtained from the phenylosazones of other sugars.

Hynd (1927a) suggested that instead of glucosone a less misleading term would be "aldo-fructose", "which would be equally correct as the compound is, at one and the same time, the keto-derivative of glucose and the aldo-derivative of fructose". Myrbäck (1939) gave 2-keto-glucose as an alternative to the term glucosone, while Ohle & Hielscher (1941), without explanation, used the name fructosone for the compound. Sowden (1947), in a plea for a systematic nomenclature for carbohydrates, pointed out, as an instance of the inadequacy of the existing system, that D-glucose phenylosazone was not essentially a derivative of D-glucose, since in the formation of an osazone there was a loss of asymmetry about C_2. The more correct general term for the compound, in his opinion, was D-<u>arabo</u>hexose phenylosazone, and for the corresponding osone, D-<u>arabo</u>hexosone.

However, the name glucosone is still widely used and accepted, and it is the intention of the author to use it throughout this present thesis.

1.2. By Decomposition of the Corresponding Phenylosazone.

1.2.1. Action of Hydrochloric Acid.

Fischer (1888) reported that treatment of D-glucose phenylosazone with fuming hydrochloric acid gave phenylhydrazine hydrochloride and a highly reactive nitrogen-free product, which could not be crystallised. He showed that the sugar, D-glucosone (oxyglucose), obtained in 40% yield, rapidly reduced Fehling's solution and, with phenylhydrazine acetate, formed D-glucose phenylosazone, both reactions occurring without the application of heat. No analysis of the osone was reported.

Fischer (1889) improved the preparative technique for D-glucosone. Purification and isolation was carried out by precipitating the osone in alkaline solution with lead hydroxide, followed by acid decomposition of the lead complex; glucosone was obtained as an almost colourless syrup. Fischer also reported the preparation of osones from the phenylosazones of galactose, sorbose, lactose, maltose, "α-acrose", "β-acrose", "formose", arabinose, and rhamnose. None of the products crystallised and all showed reducing properties similar to those of glucosone.

Hynd (1927a) further purified glucosone, prepared by the method of Fischer (1889), by repeatedly extracting it with absolute ethanol, thereby freeing the sugar from inorganic contaminants. He was unable, however, to crystallise it.

1.2.2. Action of Carbonyl Compounds.

For the improved preparation of osones (in 70% yield) from the phenylosazones of the disaccharides maltose and melibiose, Fischer & Armstrong (1902) used benzaldehyde in dilute acid medium to remove the phenylhydrazine residues, a method which Herzfeld (1895) had employed in obtaining maltose from maltose phenylhydrazone. Such a method avoided conditions liable to cause hydrolysis of either the disaccharide osazone

or osone products; in addition, the ready transformation of disaccharide phenylosazones in mineral acid solution to anhydro derivatives (Fischer, 1887a; Percival & Percival, 1937; Bayne, 1952b) would be prevented. Lewis (1909) claimed to obtain almost quantitative yields of maltosone by extending the reaction time of this method. The benzaldehyde process was used by Fischer & Zemplèn (1909) for the preparation of cellobiosone, and by Hynd (1927a) for lactosone. Similarly, Bayne (1952b) obtained an osone by decomposition of "anhydro-lactosazone", which latter compound he showed to be 3:6-anhydro-4-β-D-galactosido-D-allose phenylosazone.

Morrell & Crofts (1903a) referred to unsuccessful attempts to apply the benzaldehyde method to the decomposition of D-glucose phenylosazone, which, unlike the phenylosazones of the disaccharides, is almost insoluble in ethanol and in hot water. However, Mayer (1912) obtained glucosone, on a small scale only, by initially dissolving glucose phenylosazone in excess benzaldehyde. Hynd (1927a) modified the conditions and obtained glucosone in rather poor yield; by a similar method Percival & Percival (1935) prepared glucosone in 7% yield. By the introduction of further modifications, Bayne, Collie & Fewster (1952) increased the yield and purity of the product. Fischer & Armstrong (1902) hydrolysed maltosone, prepared by the benzaldehyde process, with an aqueous extract of brewer's yeast to give a mixture of glucosone and glucose; also melibiosone, similarly prepared, with emulsin to glucosone and galactose; Fischer & Zemplèn (1909) hydrolysed cellobiosone, prepared by the benzaldehyde method, with emulsin to glucosone and glucose. Hynd (1927a) obtained glucosone, in admixture with glucose and galactose respectively, by hot acid hydrolysis of maltosone and lactosone; hot acid hydrolysis of maltosone had previously been reported by Lewis (1909).

The benzaldehyde method was first used for the preparation of osones from the phenylosazones of pentoses, which are

soluble in ethanol and in hot water, by Fischer & Armstrong (1902). Similar preparations were later described by Morrell & Bellars (1905), but the yields of osones were very low.

Bayne (1952a) prepared D-altro-(D-gluco)-heptosone (sedoheptulosone) by decomposition of sedoheptulose phenylosazone with benzaldehyde.

Morrell & Bellars (1905) described the use of o-nitrobenzaldehyde in aqueous ethanolic solution at 125° in place of benzaldehyde for the decomposition of the phenylosazones of glucose and rhamnose, but very low yields of osones were obtained. Percival & Percival (1935) prepared glucosone by the action of p-nitrobenzaldehyde on glucose phenylosazone in a yield comparable with that obtained by Fischer (1889) using hydrochloric acid.

Brüll (1936) decomposed glucose phenylosazone with an excess of pyruvic acid and reported a 40% yield of glucosone.

Morrell & Crofts (1903a) stated that no glucosone was obtained by the action of formaldehyde on glucose phenylosazone.

1.2.3. Preparation of Osones as Intermediates in the Synthesis of L-Ascorbic Acid and its Analogues.

One of the main methods available for the synthesis of L-ascorbic acid and its analogues is the addition of hydrogen cyanide to the corresponding osone followed by hydrolysis; this method was utilised simultaneously by Ault, Baird, Carrington, Haworth, Herbert, Hirst, Percival, Smith & Stacey (1933) and by Reichstein, Grüssner & Oppenauer (1933a; 1933b) in the first syntheses of D- and L-ascorbic acid. In all syntheses carried out by this method the requisite osones have been obtained by the decomposition of the corresponding phenylosazones, although in many cases the osones were not isolated.

The following osones have been prepared by the hydrochloric acid method: D- and L-xylosone (Ault et al., 1933);

L-arabinosone (Baird, Haworth, Herbert, Hirst, Smith & Stacey, 1934); D-glucosone (Ault et al., 1933; Baird et al., 1934; Haworth, Hirst, Jones & Smith, 1934; Reichstein, Grüssner & Oppenauer, 1934); L-glucosone (Haworth, Hirst & Jones, 1937); D-galactosone (Baird et al., 1934; Haworth et al., 1934; Reichstein et al., 1934); L-gulosone (L-sorbosone) (Reichstein, et al., 1934); L-rhamnosone (Reichstein, Grüssner & Oppenauer, 1935); L-fucosone (Reichstein & Demole, 1936); D-glucohept-osone (Carpéni, 1938a).

Decomposition of the corresponding phenylosazones with benzaldehyde in aqueous ethanolic solution was employed for the preparation of the following osones: D-xylosone (Reichstein et al., 1933a; 1933b); L-xylosone (Reichstein et al., 1933b); D-arabinosone (Reichstein et al., 1934); L-arabinosone (Baird et al., 1934; Reichstein et al., 1934); D-galactosone (Baird et al., 1934); L-gulosone (Micheel, Kraft & Lohmann, 1934); L-allosone (Steiger, 1935); lactosone (Baird et al., 1934).

Smith (1946) has stated that, "In order to facilitate the isolation of the ascorbic acids it is important to obtain the osones in as pure a state as possible and this depends to a large extent upon the initial isolation of a pure osazone. Experiments have demonstrated that if the osazone is soluble in ethyl alcohol (as is usually the case with those osazones prepared from pentose sugars) it is advisable to convert the osazone into the osone by the agency of benzaldehyde. On the other hand, if the osazone is sparingly soluble in alcohol, the osone is best prepared by decomposition of the osazone with concentrated hydrochloric acid."

1.2.4. The Indirect Preparation of Ethers and Esters of D-Glucosone.

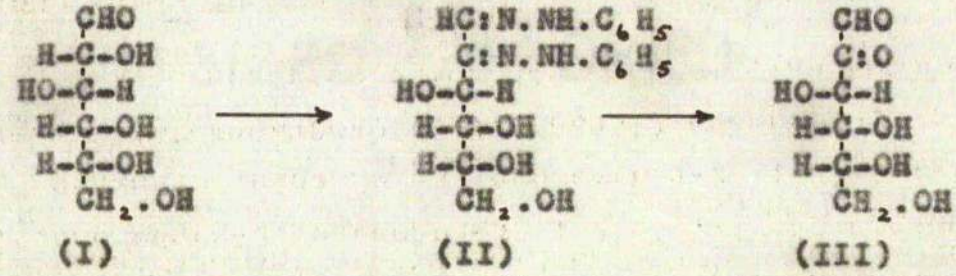
Partially methylated osones have been prepared indirectly by decomposition of the corresponding partially methylated phenylosazones. Thus, Percival & Percival (1935)

decomposed partially methylated D-glucose phenylosazone with p-nitrobenzaldehyde and obtained, they claimed, 5-O-methyl D-glucosone and 3:4:5-O-trimethyl D-glucosone. Hartley & Linnell (1940) reported that initial attempts to prepare 6-O-methyl D-glucosone by heating 6-O-methyl D-glucose phenylosazone with benzaldehyde or piperonal in aqueous ethanolic solution or with formaldehyde in aqueous solution led in each case to recovery of the unchanged phenylosazone. Decomposition of the phenylosazone was effected with hydrochloric acid; the osone was not isolated but was reduced in solution to 6-O-methyl D-fructose. Skinner (1948) prepared 3-O-methyl D-glucosone by the decomposition of 3-O-methyl D-glucose phenylosazone with benzaldehyde (c.f. Bayne, 1952a).

von Lebédev (1910) obtained D-glucosone-6-phosphate, isolated as an amorphous lead salt, by the action of hydrochloric acid on the phenylosazone of fructose-6-phosphate.

1.2.5. The Mechanisms of the Reactions.

The conversion of a sugar into the corresponding osone involves an oxidation; in the preparation of an osone from an osazone the oxidation of the parent sugar may be considered to have occurred in the formation of the osazone. Thus, Fischer (1888) visualised the conversion of D-glucose (I) to D-glucosone (III) via D-glucose phenylosazone (II) to occur as follows, all compounds being represented in the open chain form:



(I) (II) (III)

It should be noted that (II) represents the bisphenylhydrazone of (III) as well as the phenylosazone of (I), and, in fact, the editors of Beilstein give, as an alternative to "D-glucose phenylosazone", "D-glucosone bisphenylhydrazone". The correct

formulation of the reaction leading to the formation of osones from osazones is complicated by the existence of geometrical isomers of the osazones as well as the possibility of both cyclic and acyclic forms (Percival & Percival, 1935; Percival, 1936; Wolfrom, Konigsberg & Soltzberg, 1936). If, however, the osazones are written in the conventional manner as open chain bisphenylhydrazones (II) of the dicarbonyl compounds cleavage by hydrochloric acid represents hydrolysis at the azomethine linkages.

Mandl (1950) has suggested that decomposition of the osazones by benzaldehyde or its homologues is due to competition between carbonyl groups. Mandl (1950) also showed that the phenylhydrazine residues of the phenylosazones are slightly labile in acetic acid solution, and postulated that such lability would explain the mechanism of "transosazonation" (Engel, 1935) which he favoured. For example, when glucose phenylosazone was heated in acetic acid with 2:4-dinitrophenylhydrazine, glucose 2:4-dinitrophenylosazone was precipitated. Mandl proposed that slight hydrolysis of the phenylosazone occurred to give an equilibrium mixture of phenylosazone and osone; the osone was removed by immediate reaction with the 2:4-dinitrophenylhydrazine, to form practically insoluble 2:4-dinitrophenylosazone, thus disturbing the equilibrium. The reaction proceeded in this way until the entire substrate was converted to the much less soluble 2:4-dinitrophenylosazone. This hypothesis was supported by the demonstration that glucosone was produced in 4% yield by heating glucose phenylosazone in 25% acetic acid for three hours.

It is apparent that the formation of osones by the treatment of phenylosazones with benzaldehyde, in dilute acid medium, is analogous to transosazonation, the equilibrium

phenylosazone ⟶ osone + phenylhydrazine

being disturbed by removal of the phenylhydrazine as insoluble benzylidene phenylhydrazone. A similar mechanism will operate

in the decomposition of phenylosazones with hydrochloric acid, the free phenylhydrazine being removed as insoluble phenylhydrazine-hydrochloride.

1.3. By Direct Oxidation of the Corresponding Aldose or Ketose.

1.3.1. Action of Fenton's Reagent.

Fenton (1894; 1895; 1896) showed that dihydroxymaleic acid was formed by oxidation of tartaric acid with the reagent that bears his name, namely, hydrogen peroxide in the presence of ferrous sulphate. Such a reagent is in contrast to that used for the Ruff (1898) degradation, a process which utilises hydrogen peroxide in the presence of ferric salts.

Cross, Bevan & Smith (1898) applied Fenton's reagent to the oxidation of glucose, fructose, and sucrose. They found that the products reacted with phenylhydrazine and reduced Fehling's solution at ordinary temperatures, but they were unable to decide the nature of these substances.

Morrell & Crofts (1899) further investigated the oxidation of carbohydrates under these conditions and claimed to identify the first products of oxidation as osones. Thus, D-glucose gave D-glucosone, characterised by the ready formation of D-glucose phenylosazone and methylphenylosazone at room temperature, as well as the formation of precipitates with o-diamines, hydrazine hydrate, or benzoylhydrazine, also in the cold, and with aniline at slightly higher temperatures. They pointed out that Fischer (1889) considered the ready formation of a methylphenylosazone to be a characteristic test for an osone, but ignored the fact that Fischer also described the preparation of a specific methylphenylhydrazone of D-glucosone.

To avoid further degradation of the osone product Morrell & Crofts limited the action of the oxidising reagent on the sugar, but were unable to achieve complete conversion. In the case of the oxidation of D-glucose they removed unchanged sugar by fermentation with brewers' yeast, the osone being unattacked. The aqueous osone solution was then evaporated and the syrupy residue dissolved in absolute ethanol and an excess of ether added to the solution. Glucosone was precipitated

as a white hygroscopic solid which soon changed to a syrup. The oxidation of fructose was reported to be more rapid than that of glucose. Oxidation of galactose, rhamnose, and starch gave solutions that yielded phenylosazones at room temperature on addition of phenylhydrazine. The formation of an osone from arabinose was indicated by the ready preparation of arabinose methylphenylosazone under conditions which gave only a methylphenylhydrazone from arabinose. Neuberg (1902) reported the oxidation of D-arabitol with Fenton's reagent and claimed, on the basis of conversion into arabinose methylphenylosazone, the formation of D-arabulose, since under the conditions used D-arabinose yielded a methylphenylhydrazone. Morrell & Crofts (1903b) suggested that conversion into an osazone was indicative of the presence of arabinosone among the products of the oxidation. Later, Shinoda, Sato & Sato (1932) reported that oxidation of either polygalitol or styracitol with Fenton's reagent produced glucosone, characterised as glucose phenylosazone.

The osone products of the oxidation of glucose, fructose, arabinose, and rhamnose with Fenton's reagent were removed from the reaction medium by Morrell & Crofts (1900) by precipitation with lead hydroxide in alkaline solution according to the method of Fischer (1889). After acid decomposition of the lead complex the osones were characterised as their corresponding phenylosazones; the osones of arabinose and rhamnose prepared in this manner were also characterised as their respective p-bromophenylosazones (Morrell & Crofts, 1903b). Oxidation of galactose was reported (Morrell & Crofts, 1900), but no osone product was isolated, it being presumed that the oxidation proceeded too far, with the consequent production of acidic compounds. Later, Morrell & Crofts (1902) suggested that the anomalous results "were possibly due to the internally compensating positions of the hydrogen atoms and hydroxyl groups in galactose". Using neutralised hydrogen peroxide in the presence of ferrous sulphate it was shown that sucrose was first inverted, since glucosone was

was formed; similar hydrolysis of maltose and of lactose was later reported by Morrell & Bellars (1905).

The oxidation of mannose with hydrogen peroxide with the production of glucosone was also reported by Morrell & Crofts (1902). At the same time they described the preparation of 3g. of white solid by the oxidation of 60g. of D-glucose or D-fructose with Fenton's reagent, the product being isolated by the method of ether precipitation (Morrell & Crofts, 1899), after purification by the method of Fischer (1889). From the optical activity of the white solid, Morrell & Crofts were led to the conclusion that it was contaminated with a small quantity of parent carbohydrate since the osone obtained from glucose was slightly dextrorotatory, while that from fructose was moderately laevorotatory; Fischer (1889) reported that D-glucosone, prepared from D-glucose phenylosazone, was feebly laevorotatory. Morrell & Crofts showed that solutions of D-glucosone prepared by the oxidation of either glucose or fructose showed slight laevorotation after fermentation with yeast.

With regard to the mechanism of the formation of osones by this method, Morrell & Crofts (1899) considered that in the oxidation of aldoses the secondary hydroxyl group contiguous to the aldehyde group was oxidised to a carbonyl group and that the aldehyde group in the presence of the oxidising agent was not affected. In the case of fructose it was claimed that the primary hydroxyl group, adjacent to the carbonyl group, underwent oxidation to the aldehyde radical. Morrell & Bellars (1905) attempted to trace the disappearance of different sugars during oxidation with hydrogen peroxide by observing the diminution in optical rotation, and from the determination of the initial and final reducing powers of the solutions, as well as their acidities, to elucidate the stages in the oxidation process. Morrell & Crofts (1903b) had previously shown that "over-oxidation" of sugars with hydrogen peroxide led to the production of a variety of short chain acids formed by oxidative rupture of the osone

molecule. Many investigations have been carried out on the oxidation of sugars with Fenton's reagent under various conditions of pH, temperature, and concentration; however, since all workers have assumed that the primary oxidation products were osones, their results represent studies of the oxidation of osones with this reagent, and are therefore discussed under that heading in Part II, 2.2.

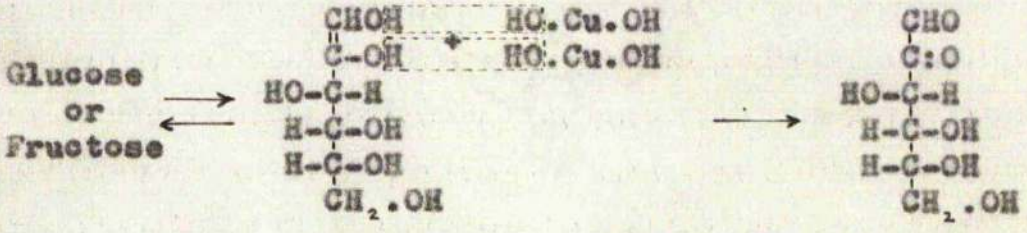
The catalytic effect of ferrous salts in the oxidation of sugars with hydrogen peroxide was ascribed, first by Spoehr (1910), and later by Küchlin (1932), to the formation of a complex involving ferrous ions and the carbonyl group of the open chain form of the sugar and its neighbouring hydroxyl group. The complex was oxidised at the hydroxyl group, the ferrous ion being converted to the ferric ion. It was suggested that, on dissociation of the oxidised complex, with the production of an osone, ferric ions might be reduced to the ferrous state at the expense of the osone. It is considered that ferric ions will not catalyse the oxidation of sugars by hydrogen peroxide, and that since ferric salts are used as catalysts in the Ruff degradation of aldonic acids to sugars containing one less carbon atom, any ferrous ions formed must be rapidly re-oxidised by hydrogen peroxide to ferric ions since no osone formation in the course of this degradation has been reported. According to Haber & Weiss (1934) ferrous salts bring about the decomposition of hydrogen peroxide into free radicals; Waters (1945) has suggested that the neutral hydroxyl radicals are the true catalysts in ferrous ion-catalysed oxidations of α-hydroxy acids to 2-keto acids.

1.3.2. Action of Cupric Acetate.

Evans, Nicoll, Strause & Waring (1928) oxidised glucose, fructose, and galactose in aqueous solution with excess cupric acetate at 50° "for the purpose of ascertaining whether the general principles underlying the mechanism of carbohydrate

oxidation in alkaline solutions are sufficient to explain the course of such oxidations in acid solutions". In the cases of glucose and fructose one of the first products of oxidation was claimed to be glucosone; the osone was not isolated, and, in fact, it was only identified in the reaction medium on the evidence of the ready formation of glucose phenylosazone without the application of heat. Evans et al. showed that after five hours oxidation 13.25% of the carbon atoms of glucose and 23.83% of those of fructose could be recovered from the reaction medium as glucose phenylosazone, formed at room temperature. No osone derivative was obtained from the oxidation of galactose, it being assumed that galactosone was further oxidised as rapidly as it was formed.

Evans and his associates visualised glucosone to be formed according to the following reactions:



(IV)

They thus regarded the intermediate enediol (IV) as an acid, and presented evidence to support this view. They showed that osone formation was accompanied by simultaneous production of formic acid, considered to arise from the oxidation of hydroxymethylene obtained by rupture of the 1:2-enediol (IV). Further oxidation produced a variety of acids by oxidative breakdown of the osone molecule; the nature and mode of formation of these products are discussed in Part I, 2.2.

Weidenhagen (1937) further investigated this type of reaction, and described a procedure for the oxidation of sugars which was a modification of a method due to Henze (1931a; 1931b) for the preparation of phenylglyoxal from benzyl alcohol. Weidenhagen found that osone formation could be made the main

reaction if, in place of water or dilute alcohol as solvent, concentrated ethanol or, preferably, methanol was used, and instead of a great excess of cupric acetate, at most 100% of the theoretical was allowed to react and that only for a very short time. In this manner 60% yields of L-gulosone and L-xylosone were obtained by the direct oxidation of L-sorbose and L-xylose, the osones being estimated by conversion into the corresponding phenylosazones. The osones were not isolated in pure state but were converted into ascorbic acid (L-gulosone via 2-oxo-L-gulonic acid and L-xylosone by the cyanhydrin method). Oxidation of other hexoses was reported to give 40% yields of the corresponding osones although a 60% yield was obtained from galactose, from which sugar previous investigators (Morrell & Crofts, 1900; Evans et al., 1928) had been unable to obtain galactosone by direct oxidation.

As a method of preparation of osones required for the synthesis of L-ascorbic acid and its analogues, the method of Weidenhagen is valuable since, for this purpose, it is not necessary to isolate the osones in a pure state. Stone (1940) patented a method of preparation of compounds of the ascorbic acid series in which the requisite osones were obtained by a modification of Weidenhagen's procedure; Saloman, Burns & King (1952), by the introduction of further modifications, prepared L-xylosone in 40-50% yield, calculated on a quantitative conversion into imino-L-ascorbic acid. Hamilton & Smith (1952) prepared D-xylosone as an intermediate, in the synthesis of D-ascorbic acid, by the method of Weidenhagen, which they considered to be "by far the best method for making osones but it does not seem to have received the recognition that it deserves". Such a statement is only valid in the case of osones to be used as intermediates in ascorbic acid syntheses, when isolation of a pure product is not essential; as a method of preparation of osones for chemical and metabolic investigations that of Weidenhagen suffers from the disadvantage common to all

methods of direct oxidation, namely, contamination of the osone with products of further oxidation.

1.3.3. Action of Other Oxidising Agents.

Morrell & Crofts (1900) described the oxidation of glucose with potassium persulphate as occurring very slowly at room temperature; the optimum temperature was reported to be 40° but the yield of osone was very small compared to that obtained by the hydrogen peroxide method previously investigated by them.

In the auto-oxidation of aqueous solutions of glucose containing quinone in the presence of light, Ciamician & Silber (1901) claimed to demonstrate the formation of glucosone, characterised as glucose phenylosazone; a similar observation was made in the auto-oxidation of aqueous solutions of glucose containing m-xylene (Ciamician & Silber, 1913). Morrell & Bellars (1905) performed auto-oxidation experiments with benzaldehyde as inductor, whereby the oxidations of glucose and fructose, in the presence of ferrous sulphate, were slightly accelerated, but the yields of osone were very poor. They also showed that radium emanations had no influence on the oxidation of carbohydrates by air in the presence of ferrous sulphate. Mayer (1911) described the formation of glucosone by the ultraviolet irradiation of aqueous solutions of glucose containing a trace of sodium carbonate, a change which did not take place without the agency of ultraviolet light.

Dixon & Harrison (1932) prepared glucosone by the oxidation of fructose, in aqueous solution, with hot selenious acid; isolation and purification was carried out after the manner of Fischer (1889), but no yield was quoted. Selenious acid had previously been used as an oxidising agent, for the preparation of substituted glyoxals, by Riley, Morley & Friend (1932), who also reported that fructose, but not glucose, was oxidised by this reagent. They did not investigate the products of the reaction.

1.3.4. Conclusions.

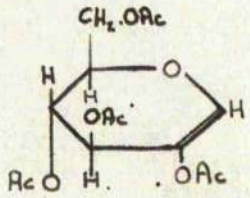
In many cases, evidence for the formation of osones by the direct oxidation of aldoses and ketoses has been inadequate, based as it has been on the conversion of the products into non-definitive osazones. As a means of obtaining pure osones, suitable for structural and metabolic investigations, all the methods described are unsatisfactory; with the exception of the method of Weidenhagen, yields of osones are very low, and in every case the osones are contaminated both with unchanged starting material and the products of further oxidation, the degree of contamination varying with each method. The separation of osones from such impurities is almost impossible, except, perhaps, by a chromatographic method (see Part II, 2.1.6.); thus, Morrell & Crofts (1900) showed that even after the isolation of the osone products by precipitation with lead hydroxide in alkaline solution (Fischer, 1889) followed by precipitation from ethanolic solution with ether they still contained unchanged parent sugar. Complete purification could, in fact, only be achieved by conversion of the osones into specific crystalline derivatives, such as methylphenylhydrazones (Fischer, 1889) or isopropylidene compounds (Bayne, Collie & Fewster, 1952), from which the osones could be readily regenerated; by such means the poor yield of osone originally obtained would be even further decreased, although the method of Weidenhagen, which can be carried out on a large scale, followed by such modes of purification, might provide a feasible preparation of pure osones (see Part II, 1.2.1.2.).

1.4. Indirect Syntheses of Substituted Osones.

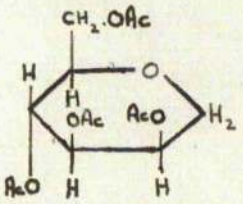
1.4.1. O-Acetyl and O-Benzoyl Derivatives of Osones.

Maurer & Mahn (1927), in the first of a series of papers, described the preparation of two new anhydrosugars by the action of diethylamine and other aliphatic secondary amines on 2:3:4:6-tetra-O-acetyl-D-glucosyl bromide and 2:3:4:6-tetra-O-acetyl-D-galactosyl bromide respectively.

Maurer (1929) reported an improved preparation of the anhydrosugar derived from tetra-O-acetyl-D-glucosyl bromide. He formulated the product as (V) and named it "2:3:4:6-tetra-acetyl-1:2-glucoseen", in accordance with the nomenclature of Helferich & Himmen (1928), who had synthesised and named "tetra-acetyl-(5:6)-glucoseen".



(V)

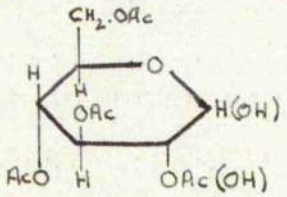


(VI)

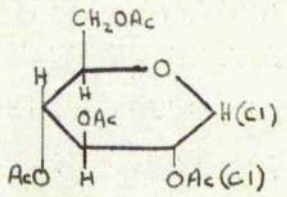
Catalytic hydrogenation of (V) was achieved by Zervas (1930) with the production of the dihydro derivative (VI), identified as tetra-O-acetyl styracitol (tetra-O-acetyl-1:5-anhydro D-mannitol); the other predicted 2-epimer, tetra-O-acetyl polygalitol, was found in much smaller amounts by Richtmyer, Carr & Hudson (1943) after catalytic hydrogenation of (V). It is interesting to recall that Shinoda, Sato & Sato (1932) claimed to obtain D-glucosone by the oxidation of styracitol or polygalitol with Fenton's reagent (see Part I, 1.3.1.).

Maurer (1929) showed that treatment of (V) in dry ether with chlorine produced a mixture of isomeric dichloro derivatives which could not be crystallised. Addition of silver carbonate and a few drops of water to the chlorination products in ether caused evolution of carbon dioxide and separation of a crystalline compound. From the observations that the latter reduced

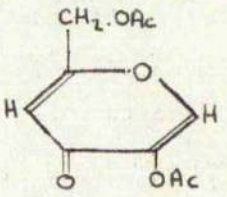
Fehling's solution in the cold, decolorised potassium permanganate in sodium carbonate solution, exhibited mutarotation, and, after short treatment with alkali, gave D-glucose phenylosazone with phenylhydrazine at room temperature the compound was formulated as 2:3:4:6-tetra-O-acetyl D-glucosone hydrate (VII). This was the first recorded observation of mutarotation of a solution of an osone or substituted osone.



(VII)



(VIII)



(IX)

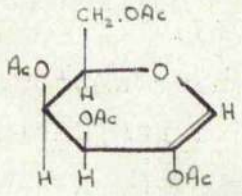
One of the dichloro derivatives, formulated as (VIII), of tetra-O-acetyl-2-oxyglucal* was later crystallised (Maurer, 1930) and this was reported to give (VII) in 90% yield when treated with silver carbonate. With acetic anhydride in pyridine at 0° tetra-O-acetyl D-glucosone hydrate gave a crystalline product which was identified as di-O-acetyl kojic acid (IX), which, on solution in methanolic ammonia, gave successively mono-O-acetyl kojic acid and the free acid, 5-hydroxy-2-hydroxymethyl-γ-pyrone. In this manner glucose was converted into kojic acid by purely chemical means; hitherto the acid was only obtained as a metabolic product of many fungi (mainly Aspergilli) and certain bacteria (for reviews see Barham & Smits, 1934; Foster, 1949).

Maurer & Müller (1930) described the preparation of tetra-O-acetyl-2-oxy-D-galactal (X). Chlorination of (X), followed by treatment with moist silver carbonate gave, they claimed, crystalline tetra-O-acetyl D-galactosone hydrate (XI),

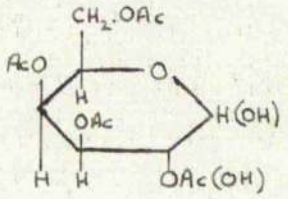
---

* The designation "1:2-glucoseen" previously proposed (Maurer, 1929) was abandoned at the suggestion of M. Bergmann and the editors of Beilstein, although this terminology is still commonly used.

which showed properties similar to those of (VII); (XI) gave D-galactose phenylosazone with phenylhydrazine at room temperature, after short treatment with alkali, and with acetic anhydride in pyridine kojic acid was formed.



(X)

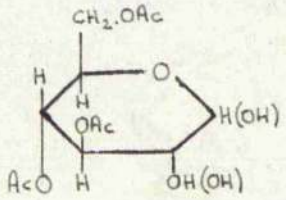


(XI)

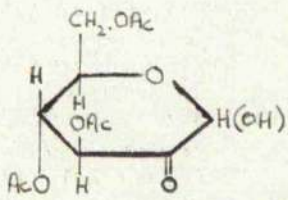
Maurer & Plötner (1931) carried out similar investigations on hepta-O-acetyl-2-oxycellobial and on hepta-O-acetyl--2-oxy-gentiobial. They claimed that the former gave one crystalline dichloride which, with excess silver acetate in hot acetic acid, yielded crystalline nona-O-acetyl cellobiosone hydrate, but did not react with moist silver carbonate; the mixture of non-crystalline dichloro derivatives, however, did react, with the production of crystalline hepta-O-acetyl cellobiosone hydrate. The latter compound, after alkaline hydrolysis, did not form cellobiose phenylosazone with phenylhydrazine. Maurer & Plötner reported that neither nona- nor hepta-O-acetyl cellobiosone hydrate, with acetic anhydride in pyridine, gave a kojic acid derivative , but, instead, hepta-O-acetyl cellobiosone. They postulated that kojic acid formation was precluded by the glucosidic linkage on C_4, which, being stable to acetic anhydride, prevented the formation of a double bond between C_4 and C_5; instead, the excess acetic anhydride converted the hydrated group at C_2 into a free carbonyl form. It was claimed that the non-crystalline products of chlorination of hepta-O-acetyl-2-oxygentiobial, with moist silver carbonate, yielded an osone product, identified qualitatively, which, with acetic anhydride in pyridine, was converted into a kojic acid derivative, again identified qualitatively. This was explained on the grounds that in gentiobiosone the glucosidic residue is

attached to C_6, i.e. on the side chain of the pyranose ring, and thus would not hinder the formation of a γ-pyrone structure.

Maurer & Petsch (1931) reported that reaction of the di-chloro-tetra-O-acetyl-2-oxy-D-glucal (VIII) with sodium or ammonium bicarbonate instead of silver carbonate produced a crystalline compound which showed chemical properties similar to those of (VII) and exhibited mutarotation. They formulated this compound as 3:4:6-tri-O-acetyl D-glucosone hydrate (XII). It was considered that an acetyl group was absent from C_2 since the compound was not a stable hydrate, the elements of one molecule of water being lost on storage *in vacuo* over phosphorus pentoxide to form a syrup, formulated as 3:4:6-tri-O-acetyl D-glucosone (XIII), having chemical properties similar to those of (XII).



(XII)



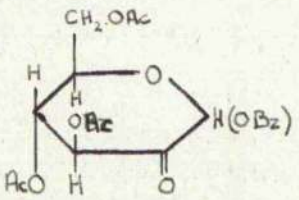
(XIII)

No mutarotation was observed in aqueous ethanolic solutions of (XIII) (c.f. XII), which, in view of the proposed structure, is surprising; no positive evidence for the existence of a free carbonyl group at C_2 was presented. Under the same conditions tetra-O-acetyl glucosone hydrate was not dehydrated. Maurer & Petsch were unable to decide whether the readily eliminated molecule of water of (XII) was present as water of crystallisation or of structure, but suggested that structures (XII) and (XIII) might be present, in equilibrium, in solution; the lack of mutarotation of (XIII), however, suggests that the dehydration is not reversible.

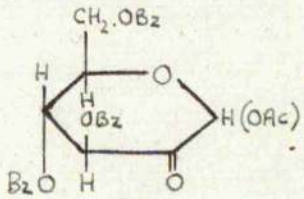
(VII), (XII), and (XIII) were shown to be transformed into di-O-acetyl kojic acid merely by dissolving them in pyridine or aqueous pyridine (see Part I, 2.2.). The structures of these three compounds were confirmed by determination of the

number of free hydroxyl groups by the Tschugaev-Zerewitinov method.

Maurer & Petsch (1933) reported that 1-O-benzoyl-3:4:6--tri-O-acetyl D-glucosone (XIV), prepared by the action of benzoyl chloride and pyridine in cold chloroform on tetra-O--acetyl glucosone hydrate, reduced Fehling's solution and decolorised potassium permanganate in acetone but was unaffected by hot pyridine, and not debenzoylated by hydrogen bromide.



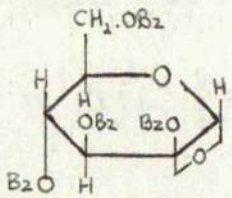
(XIV)



(XV)

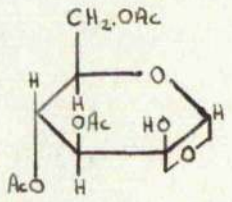
Tetra-O-benzoyl-2-oxy-D-glucal was reported to give a crystalline dichloride which formed di-O-benzoyl kojic acid with hot pyridine; with sodium acetate in hot acetic anhydride the dichloro derivative gave 1-O-acetyl-3:4:6-tri-O-benzoyl D-glucosone (XV), which was unchanged by the action of pyridine. No direct evidence for the presence of a free carbonyl group in either (XIV) or (XV) was presented.

The mixture of non-crystalline dichlorides of tetra-O--benzoyl-2-oxy-D-glucal, with sodium bicarbonate, gave a compound containing four benzoyl groups which reduced Fehling's solution in aqueous acetone and decolorised permanganate solution, but did not exhibit mutarotation; with pyridine, di-O-benzoyl kojic acid was formed but the compound was stable towards acetic anhydride and acid reagents. The compound was named 2:3:4:6-tetra-O-benzoyl D-glucosone and formulated as (XVI).

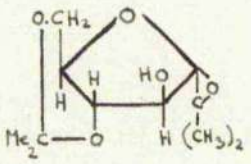


(XVI)

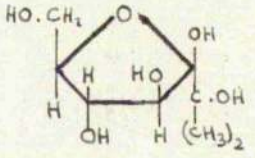
The compound (XVI) might be better described as 2:3:4:6-tetra-O-benzoyl-1:2-anhydro D-glucosone hydrate, and if this structure is possible the molecule of water associated with C_2 in the analogous acetyl derivatives (VII) and (XII) is certainly not one of crystallisation. That the ethylene oxide ring in (XVI) was easily opened was shown by the ready transformation into di-O-benzoyl kojic acid and the formation of a tri-O-benzoyl glucose phenylosazone with phenylhydrazine in 75% acetic acid. Maurer & Petsch (1933) also obtained a series of partially acetylated glucose phenylosazones from intact and partially hydrolysed tri-O-acetyl D-glucosone hydrate (XII). The presence of an ethylene oxide ring in (XVI) would also explain the observed lack of mutarotation by the compound. It is suggested by the present author that the dehydration product of tri-O-acetyl D-glucosone hydrate (XII), formulated by Maurer & Petsch (1931) as (XIII), which also showed no mutarotation, is better represented by the structure (XVII), such a structure being in line with the proposed (XVI).



(XVII)



(XVIII)

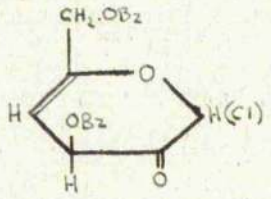


(XIX)

A 1:2-anhydro ring was considered by Ohle & Hecht (1930) to be present in the acetonation product (XVIII) of 1:1-di-C-methyl fructose (XIX) (see Part I, 1.4.2.).

Maurer & Böhme (1936) reported that tetra-O-benzoyl D-glucosone, with acid chlorides in acid solution or with dry hydrogen chloride in ether, added on halogen acid with the simultaneous production of two moles of benzoic acid. They considered that since the reaction took place in acid chlorides it was not a hydrolysis, and that the hydroxyl groups required for the formation of the benzoic acid were supplied by dehydration of the sugar with the consequent production of

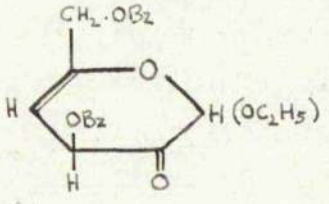
1-C-chloro-3:6-di-O-benzoyl D-glucosone-4:5-ene (XX).



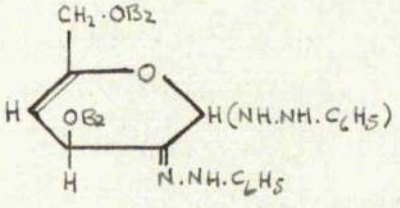
(XX)

(XX) reduced Fehling's solution readily, decolorised bromine water, and reacted with two moles of phenylhydrazine to form a phenylosazone, with the loss of the halogen atom. A vigorous reaction was reported to occur with pyridine but no homogeneous product was isolated; by the action of sodium acetate in acetic anhydride (XX) was transformed into di-O-benzoyl kojic acid.

Maurer & Böhme were unable to replace the halogen atom of (XX) with a hydroxyl group but on attempted recrystallisation of the compound from ethanol they reported that there was a quantitative replacement of the chlorine atom by an ethoxyl group to form a "glucosonide" (XXI).



(XXI)



(XXII)

Analogous crystalline compounds were obtained with methanol, benzyl alcohol, aniline, and ethane thiol; all reduced Fehling's solution only after vigorous boiling, and gave the same di-O--benzoyl phenylosazone, formulated as (XXII). In view of the proposed structures for these "glucosonides", e.g. (XXI), the behaviour of them towards Fehling's solution is surprising; thus, Militzer (1944) has shown 2-oxo-D-gluconic acid, a compound also considered to possess a free, or at least potentially free, carbonyl group at C_2, to reduce Benedict's reagent without the application of heat; similar reactivity was reported to be shown

by 5-oxo-D-gluconic acid. The presence of a free carbonyl group in the "glucosonides" was considered by Maurer & Böhme to be established by the formation of semicarbazones, together with the fact that the compounds did not exhibit mutarotation; however, if such a group were present it might be expected that reaction of 1-C-chloro-3:6-di-O-benzoyl D-glucosone 4:5-ene (XX) with ethane thiol would have produced a bisdimercaptal. It was reported that the "glucosonides" were not affected by pyridine but that with sodium acetate in acetic anhydride di-O-benzoyl kojic acid was formed. It was claimed, on the evidence of polarimetric observations, that the change (XX) → (XXI) was accompanied by a Walden inversion, the reaction being comparable to the formation of glycosides from acyl-glycosyl halides.

Maurer & Böhme found it impossible to present direct proof of the presence and position of the double bond in (XXI). Debenzoylation of the "glucosonides" gave strongly reducing syrups; the enhanced reducing power was attributed to the presence of the free hydroxyl group on C_3, the glycosidic linkage being considered to remain intact since the products did not exhibit mutarotation. The glycosidic linkage in (XXI) was reported to be stable not only towards alkalis but also towards acids; this latter stability is in direct contrast to the instability towards acids of the glycosides of the simple sugars. Treatment of the "ethyl glucosonide" with pyridine for three days gave an optically inactive compound containing one less benzoyl group, while catalytic hydrogenation gave a product which could not be acetylated. This was in contrast to tetra-O-acetyl D-glucosone hydrate (VII) which, on catalytic hydrogenation in acid solution, took up one mole of hydrogen with the production of a crystalline compound which did not exhibit mutarotation and which formed an acetyl derivative.

The published work of Maurer and his associates which has been reviewed briefly in the preceding pages may be

criticised on a number of general points apart from those already noted. These workers presented no absolute proof that their products of indirect synthesis were indeed osone derivatives and the characteristic reaction of these products, namely, conversion into kojic acid, was not shown to take place with unsubstituted osones prepared by conventional methods. Many of the proposed structures, particularly those of the "glucosonides", are not entirely compatible with the reported properties and reactions of these compounds; the suggestion that certain of these compounds were present in solution as equilibrium mixtures of hydrated and non-hydrated forms was not substantiated.

Stacey & Turton (1946) claimed to prepare tetra-O--acetyl D-glucosone hydrate by oxidation of tetra-O-acetyl-2--oxyglucal with perbenzoic acid; their product differed considerably from that of Maurer (1929) with regard to melting point and, in addition, it did not exhibit mutarotation, but showed the same chemical properties. They considered that their compound was undeniably an osone derivative since, after deacetylation, it could be converted into D-glucoascorbic acid by the cyanhydrin method; bromine oxidation followed by acid treatment produced a compound which exhibited an ultraviolet absorption similar to that of D-araboascorbic acid. Stacey & Turton prepared a crystalline monomethyl ether and a syrupy dimethyl ether of tetra-O-acetyl glucosone hydrate. By titration with dilute sodium carbonate solution they showed tetra--O-acetyl glucosone hydrate to possess an incipiently ionic hydrogen atom which they considered to be associated with the hydrated carbonyl group at C_2; they specified the possible reasons for the hydration of this carbonyl group. Stacey & Turton demonstrated, by spectrophotometric methods, that tetra--O-acetyl glucosone hydrate was converted into a kojic acid derivative by dilute alkali and proposed a mechanism for the transformation differing from that put forward previously by

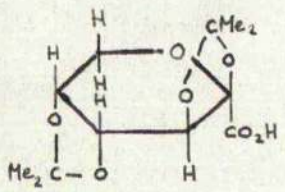
Maurer & Petsch (1931) and Isbell(1944) (see Part I, 2.2.). Stacey & Turton made no attempt to confirm these observations with unsubstituted glucosone.

1.4.2. 1-C-Methyl and 1-C-Phenyl D-Glucosone.

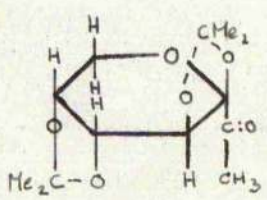
Paal & Hornstein (1906) claimed to obtain 1:1-di-C--phenyl D-glucitol by the action of phenylmagnesium bromide on tetra-O-acetyl D-gluconolactone; later, analogous derivatives of D-galactitol (Paal & Wiedenkoff, 1906) and L-arabitol (Paal & Kinscher, 1911) were prepared. Paal & Zahn (1907) reported that treatment of methyl D,L-glycerate with phenyl magnesium bromide followed by hydrolysis gave 1:1-di-C-phenyl D,L-glycerol.

In 1930 Ohle initiated a series of extensive studies on the reactions of Grignard reagents with a variety of carboxylated sugar derivatives. A study of his published work is relevant to this thesis since he claimed that substituted osones were among his products.

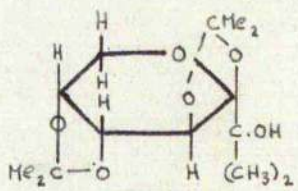
Ohle & Hecht (1930) showed that when 2:3-4:5-di-O-iso--propylidene-2-oxo- D-gluconic acid (XXIII) reacted with four moles of methylmagnesium iodide two products were obtained in a ratio of seven to one, 1-C-methyl-2:3-4:5-di-O-isopropylidene D-glucosone, formulated as (XXIV), and 1:1-di-C-methyl-2:3-4:5--di-O-isopropylidene D-fructose (XXV).



(XXIII)



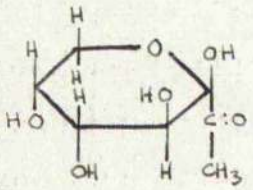
(XXIV)



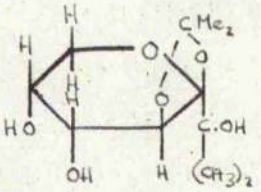
(XXV)

The further reaction of the Grignard reagent with (XXIV) produced (XXV) as did its action on the methyl ester of (XXIII). (XXIV) and (XXV), which were obtained as syrups, were separated by fractional distillation. Hydrolysis of (XXIV) with

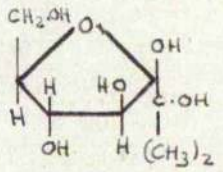
2N-sulphuric acid gave syrupy 1-C-methyl D-glucosone (XXVI), which was characterised as a crystalline phenylosazone, formed only on heating with three moles of phenylhydrazine acetate. This last reaction is in contrast to free glucosone which forms glucose phenylosazone with two moles of phenylhydrazine at room temperature. Similar hydrolysis of (XXV) yielded a mixture of products postulated to be 1:1-di-C-methyl-2:3-O-isopropylidene D-fructose (XXVII) and 1:1-di-C-methyl D-fructose (XXVIII), formulated as a fructofuranose derivative ; both compounds were obtained crystalline.



(XXVI)

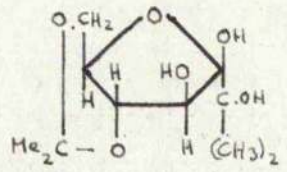


(XXVII)

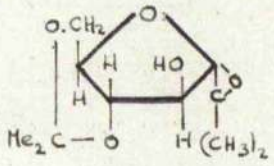


(XXVIII)

Reacetonation of 1:1-di-C-methyl D-fructose gave derivatives formulated as 1:1-di-C-methyl-4:6-O-isopropylidene D-fructofuranose (XXIX) and 1:1-di-C-methyl-4:6-O-isopropylidene-1:2--anhydro D-fructofuranose (XXX); this is surprising, since a ring shift followed by formation of (XXV) might be expected.



(XXIX)

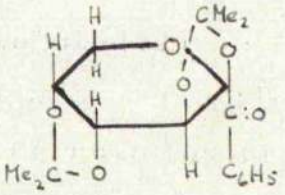


(XXX)

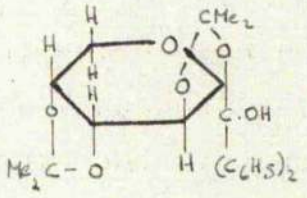
The evidence for the proposed structures of these products of hydrolysis was inadequate. Ethyl, propyl, isopropyl, butyl and isobutyl derivatives corresponding to (XXIV) and (XXV) were also prepared by the action of the appropriate Grignard reagent on the methyl ester of (XXIII).

Ohle & Blell (1931) carried out a more extensive investigation of compounds of type (XXIV). When six moles of phenylmagnesium bromide reacted with (XXIII) two products,

formulated as 1-C-phenyl-2:3-4:5-di-O-isopropylidene D-glucosone (XXXI) and 1:1-di-C-phenyl-2:3-4:5-di-O-isopropylidene D-fructose (XXXII), were obtained; these two derivatives were separated by fractional distillation and both were obtained crystalline.

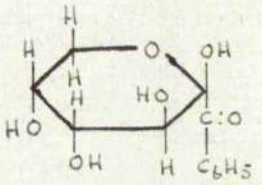


(XXXI)

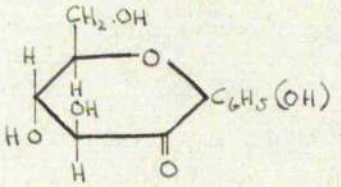


(XXXII)

When (XXXI) was hydrolysed with 2N-sulphuric acid in propanol reducing 1-C-phenyl D-glucosone resulted which was crystalline and was formulated as (XXXIIIa) or (XXXIIIb); a cyclic structure was considered to be present since the compound mutarotated in pyridine solution. That a true mutarotation was observed is open to doubt, the free osones ("free"with regard to C_1 and C_2), at least, being labile in pyridine, as shown by Maurer (1930), and in addition no mutarotation was observed in aqueous solution.



(XXXIIIa)

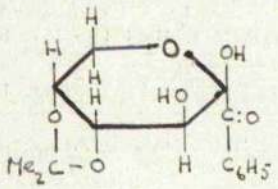


(XXXIIIb)

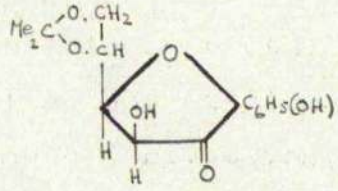
Ohle & Blell presented no evidence as to the ring size in this compound or as to the position of ring attachment. It has been suggested by Bonner (1951) that the carbonyl group at C_2 would be the one involved in the furanose or pyranose ring system in 1-C-phenyl D-glucosone owing to a possible deactivating effect of the phenyl group attached to the carbonyl group at C_1.

Acetonation of 1-C-phenyl D-glucosone yielded a reducing monoisopropylidene derivative formulated as (XXXIVa) or (XXXIVb). If the so-called 1-C-phenyl D-glucosone is indeed a true osone

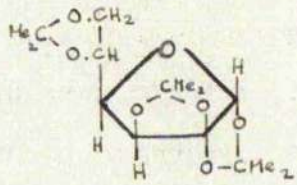
represented by (XXXIIIb), it might be expected to give a tri-isopropylidene hydrate analagous to that (XXXVI) obtained from D-glucosone by Bayne, Collie & Fewster (1952). (XXXIIIa) would at least be expected to be reconverted into the reducing 2:3-4:5-di-O-isopropylidene derivative (XXXI) on acetonation.



(XXXIVa)

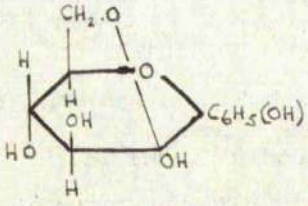


(XXXIVb)

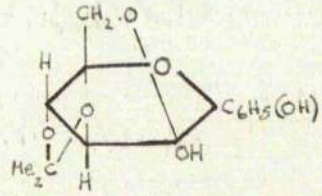


(XXXV)

Ohle & Blell reported that 1-C-phenyl D-glucosone gave a tetra-acetate on treatment with acetic anhydride in pyridine and proposed alternative acetylated structures derived from (XXXIIIa) and (XXXIIIb) for the compound; the stability of a true osone derivative under the conditions of acetylation used is to be doubted. The fact that the tetra-acetate gave no hemiacetal bromide or chloride under the usual conditions, however, also suggested to Ohle & Blell the possibility of an acyclic modification, but experimental evidence for deciding this point was not offered. The formation of a tetra-acetate satisfies structures (XXXIIIa) and (XXXIIIb), containing one lactol ring, and also an acyclic modification. It was originally suggested by Hynd (1927a) that the osones might be proved to contain two lactol rings and, in fact, such a form (XXXVI) of 1-C-phenyl D-glucosone could account for the formation of a reducing monoisopropylidene derivative, which might be formulated as (XXXVII), as well as the production of a tetra-acetate. Furanose modifications of (XXXVI) and (XXXVII) are also possible.



(XXXVI)

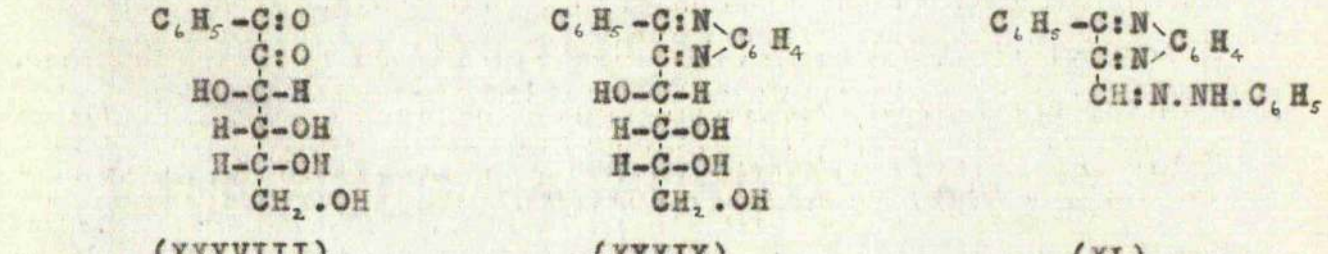


(XXXVII)

Ohle & Blell (1931) also prepared a phenylhydrazone from 1-C-phenyl D-glucosone but were unable to obtain a phenylosazone. This is surprising since ready formation of osazones is considered to be characteristic of osones (c.f. difficulty of formation of an osazone from 1-C-methyl D-glucosone). This apparent lack of reactivity of these compounds cannot be explained in terms of steric hindrance since both phenylglyoxal and phenylpyruvic aldehyde readily yield phenylosazones (Fischer, 1887; Müller & Pechmann, 1889).

Ohle & Blell (1931) showed that when benzylmagnesium bromide reacted with (XXIII), 1:1-di-C-benzyl-2:3-4:5-di-O-isopropylidene D-fructose was obtained, but a product analagous to 1-C-methyl and 1-C-phenyl D-glucosone was not isolated.

Ohle & Hielscher (1941) presented further evidence to substantiate the proposed structure of the 1-C-phenyl derivative to which they gave the alternative name "1-phenyl-D-fructosone". Reaction with o-phenylenediamine produced a crystalline compound to which they assigned the structure (XXXIX), i.e. 2-phenyl-3-[D-arabotetrahydroxybutyl]-quinoxaline, the substituted osone reacting in the open chain form (XXXVIII). The formation of such quinoxaline derivatives has been used for the detection of osones (Fischer, 1889; Ohle, 1934). (XXXIX), on treatment with hot phenylhydrazine, yielded crystalline 2-phenyl-quinoxaline-3-aldehyde phenylhydrazone (XL).



(XXXVIII) (XXXIX) (XL)

No evidence was presented to support the formulation of a free carbonyl group in either 1-C-methyl or 1-C-phenyl D-glucosone, and this, together with the difficulty of phenylosazone formation ~~xx~~ and results of reacetonation, would suggest that

without further investigation these compounds cannot be considered as true osone derivatives.

1.5. The Formation of Osones as Intermediates.

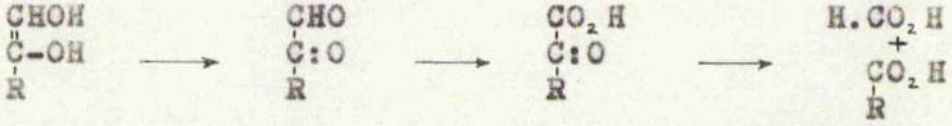
Osones have been postulated as intermediates in a great variety of reactions in the carbohydrate field but in very few cases have these claims been substantiated by isolation and characterisation of such intermediates, evidence for their formation being of an indirect nature. In this section a number of such reactions are reviewed.

1.5.1. Oxidation of Sugars.

The preparation of osones by the direct oxidation of sugars by reagents such as that of Fenton, cupric acetate, selenious acid, etc. represents the utilisation of series of reactions in which osones are initial products, the oxidant being allowed limited action thereby making the osones the main product. The nature and mechanism of formation of the products of further oxidation of osones are discussed in Part I, 2.2. It should be noted that osones have been shown to be formed during the oxidation of sugars with hydrogen peroxide only in the presence of ferrous salts. Thus, Spoehr (1910) identified a number of the acid products of the oxidation of hexoses with alkaline solutions of hydrogen peroxide and regarded various dienols, and not osones, as intermediates; Jolles (1911) reported the formation of glucuronic acid by oxidation of glucose with hydrogen peroxide at 37° in the absence of catalyst. Payne & Foster (1945) showed that at low temperature, in the absence of catalyst, the oxidation by peroxide was very slow, while at high temperatures the main product was carbon dioxide and formaldehyde which on further oxidation gave formic acid and molecular hydrogen; no osones were reported as intermediates. The Ruff degradation of aldonic acids has been discussed previously (Part I, 1.3.1.).

In dilute alkaline solution, air, or preferably, oxygen has been shown to degrade the sugars to aldonic acids containing one carbon atom less than the substrate sugar. Relatively high

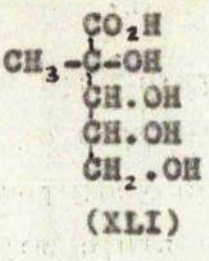
yields of acids have been obtained from aldoses (Nef, 1914; Spengler & Pfannenstiel, 1933, 1935; Isbell, 1942). Ketoses, although more liable to further degradation, act similarly (Dalmer & Heyns, 1939; Richtmyer, Hann & Hudson, 1939a, 1939b; Isbell, 1942) and 2-oxo acids corresponding to the substrate ketoses have been isolated as intermediates. The formation of the 2-oxo acids has been regarded as indicating that the oxidation proceeds via the corresponding osone, possibly formed from the enediol:



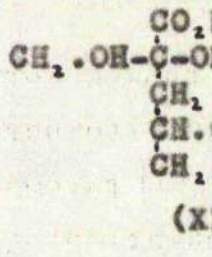
The oxidation of aldoses in dilute alkaline solution in the presence platinum or palladium catalysts with the formation of the corresponding aldonic acids has been reported (Busch, 1941; Heyns & Heinemann, 1947), while under similar conditions ketoses yield the corresponding 2-oxo acids (Heyns, 1947). In neutral solution, in the presence of platinum catalyst, the process of oxidation of aldoses is considered to be one of dehydrogenation, with the production of the corresponding saccharic acids (Glattfield & Gershon, 1938). For ketoses the reaction appears to proceed differently; thus, D-fructose has been shown to give D-arabinose, D-glucosone and 2-oxo-D-gluconic acid being regarded as intermediates.

1.5.2. Action of Strong Alkalis on Sugars.

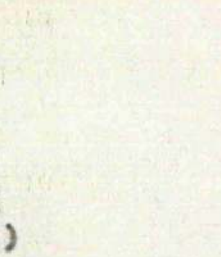
Scheibler (1880) and Kiliani (1882) showed that in strong alkaline solution extensive rearrangement of the sugars occurred, leading to the formation of saccharinic acids (XLI). Kiliani (1885) reported that the action of lime on maltose, lactose or galactose led to the production of isosaccharinic acid, formulated as (XLII). Still a third type of acid was isolated by Kiliani & Naegell (1902) from the products of the action of alkalis on galactose and lactose, termed metasaccharinic acid and formulated as (XLIII).



(XLI)



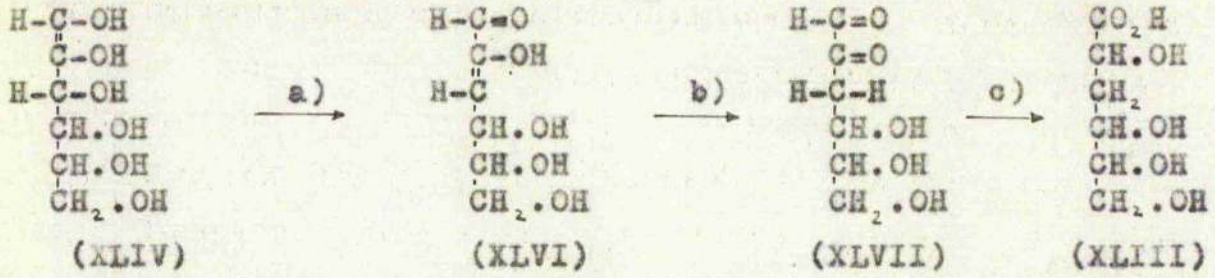
(XLII)



(XLIII)

The mechanism of the formation of these various acids is still not fully understood. Nef (1907b) postulated a progressive migration of the keto group of the sugar along the carbon chain, via enediol structures, with the formation of 2- and 3-ketohexoses. He assumed that these ketoses, by internal oxidation and reduction, formed deoxy-dioxo compounds which underwent benzilic acid rearrangements. According to Nef's concept, the deoxy-dioxo compounds were formed through intermediate methylenic compounds containing an active bivalent carbon atom. Isbell (1944) disagreed with such an explanation, although accepting the occurrence of benzilic acid rearrangement. Isbell considered that the formation of the various acids could be explained in accordance with the concept of consecutive electron displacement by the following reactions: a) ionisation of a hydrogen atom of an enediol followed by elimination of a hydroxyl group. b) "Ketonisation" with the formation of a deoxy-dioxo compound, all of which may be considered as osone derivatives; c) a rearrangement of the benzilic acid type, giving the respective acid. The latter rearrangement was presumed to take place through an intermediate ion formed either by addition of a hydroxyl ion, or by loss of a proton from the hydrated carbonyl group of the osone-like intermediate. The following reactions were proposed for the formation of the individual acids, commencing with either the 1:2-(XLIV) or 2:3-enediols (XLV) formed by the initial action of the alkali on the sugar:

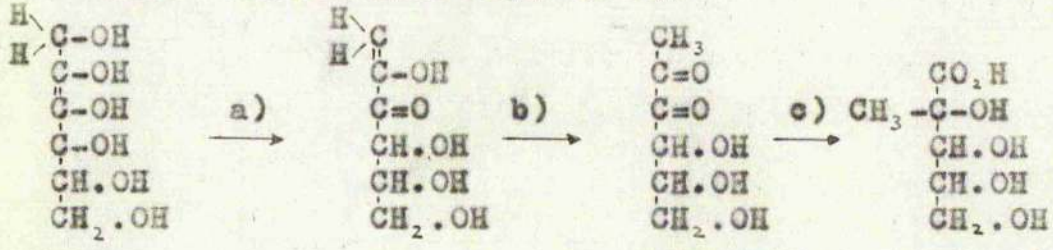
(i) Metasaccharinic acid (XLIII).



(XLIV) (XLVI) (XLVII) (XLIII)

The intermediate (XLVI) represents the 2:3-enol of the open chain 3-deoxy-osone (XLVII). A benzilic acid rearrangement of, for example, D-glucosone to D-gluconic acid under alkaline conditions has not been reported.

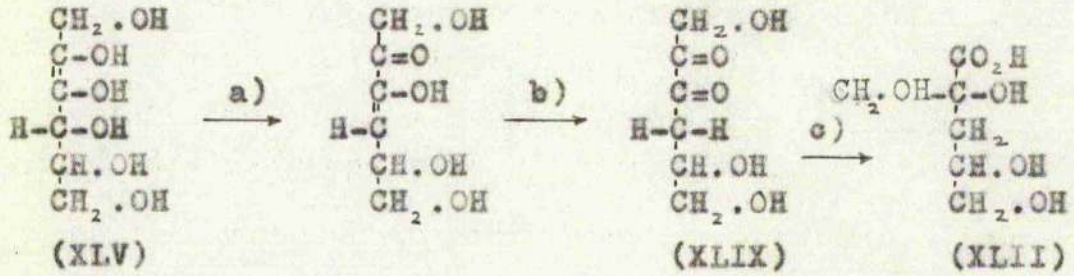
(ii) Saccharinic acid (XLI).



(XLV) (XLVIII) (XLI)

It should be noted that the hypothetical intermediate (XLVIII) is the acyclic form of a 1-C-methyl pentosone analogous to the 1-C-methyl D-glucosone of Ohle & Hecht (1930).

(iii) Isosaccharinic acid (CLII).



(XLV) (XLIX) (XLII)

(XLIX) may be considered to be a 1-C-hydroxymethyl pentosone.

Alternative mechanisms, involving the intermolecular recombination of fragments of the original sugar have been largely disregarded on account of the failure to observe formation of higher-carbon acids from the action of alkali on lower-carbon sugars. Recently, however, Sowden & Kuenne (1953), using ^{14}C labelled hexoses, have shown that the branched-chain saccharinic acids and the straight-chain metasaccharinic acids

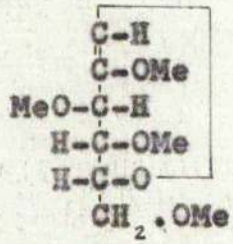
are formed by different general mechanisms. Their results on the former are incompatible with the benzilic acid rearrangement as postulated by Nef and Ebell, but are compatible with a recombination mechanism involving sugar fragments. On the other hand, their results for the straight chain acids are in agreement with such benzilic acid rearrangements.

The degradation of reducing sugars by strong solutions of alkaline hydroxides, under more drastic conditions, to a large number of acids of varying chain length was fully investigated by Nef and his coworkers (Nef,1907a, 1907b, 1910, 1914; Lewis, 1909; Anderson, 1909; Spoehr, 1910). As a result of these investigations, Nef (quoted by Anderson, 1909) concluded that one of the initial reactions that occurred in such fragmentation of the hexoses was oxidation to the hexosones, which then gave, by a benzilic acid rearrangement, the corresponding pairs of hexonic acids. Rupture of the carbon chain was considered to occur via 2:3- and 3:4-enediols formed either from the osones or from the ketoses, which could be formed from the aldoses by the action of the alkali. Rupture of these ene-diols produced tetroses and trioses respectively which were then oxidised to the corresponding osones which in turn underwent benzilic acid rearrangement or were further degraded. Such a series of reactions was proposed to explain the formation from D-galactose of D-galactonic, D-talonic, D-erythronic, L-threonic, D- and L-glyceric, glycollic, oxalic, formic, and carbonic acids by the action of Fehling's solution. The degradation of sugars by the action of alkali has been twice reviewed by Evans (1929, 1942).

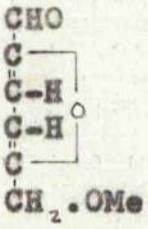
1.5.3. Formation of Furfural, 5-Hydroxymethylfurfural and Laevulinic Acid.

Following a suggestion of Raymond (1938) that "tetra-O-methyl-1:2-hydroxyglucal" (L) was possibly an intermediate in the epimeric conversion of tetra-O-methyl glucose into tetra-O-methyl mannose, Wolfrom, Wallace & Metcalf (1942) found that in aqueous

acid solution at room temperature the tetra-O-methyl 2-hydroxyglucal was smoothly converted into 5-methoxymethyl: furfural (LI)

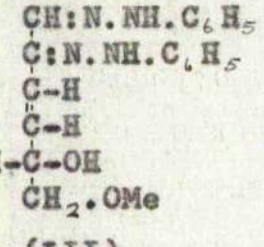


(L)

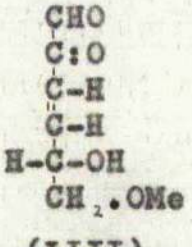


(LI)

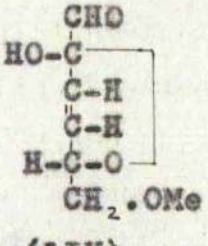
By allowing the reaction to proceed for a short time only Wolfrom et.al. were able to isolate an intermediate as its crystalline phenylosazone to which they assigned the structure (LII), from which they concluded that the intermediate in the formation of 5-methoxymethylfurfural was the unsaturated 6-O-methyl osone (LIII)



(LII)

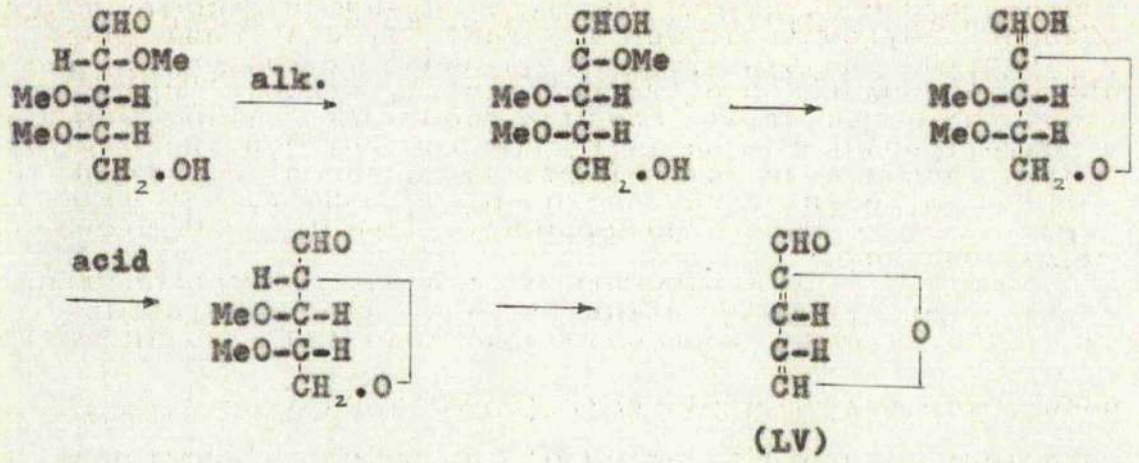


(LIII)



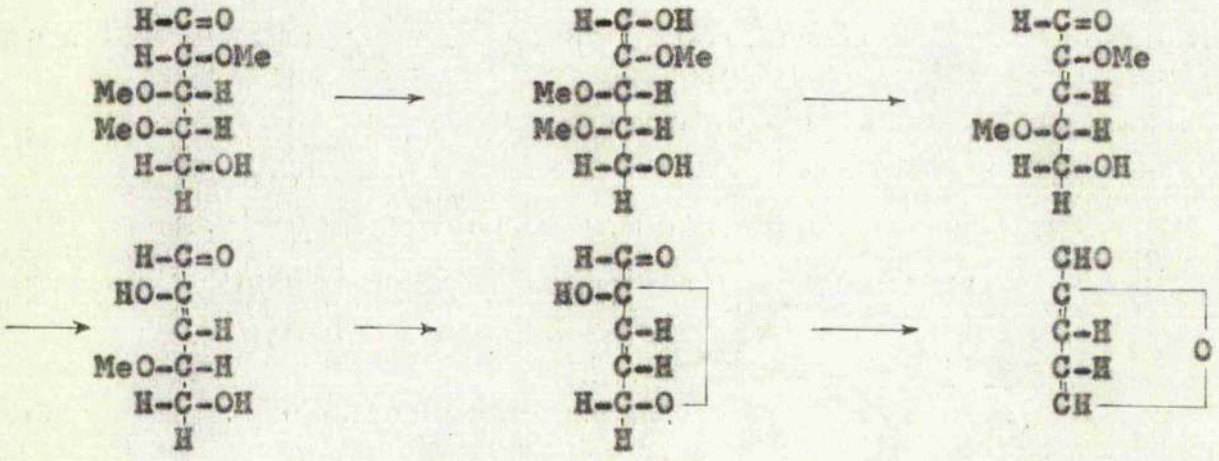
(LIV)

This was the first intermediate to be isolated in the formation of a furan compound from a carbohydrate. Apart from the analytical data and confirmation of the presence of one free hydroxyl group in (LIII), however, no further evidence was given to support the structures (LII) and (LIII) although the reaction has been considered in terms of consecutive electron displacements by Isbell (1944) on the basis of the intermediate being (LIII) which he represented as (LIV), containing a 2:5-furanose ring. In addition, such a reaction is in line with and clarifies the observation of Neher & Lewis (1931) that enols of methylated pentoses, on treatment with dilute acid at room temperature, yield furfural (LV). In the case of tri-O-methyl L-arabinose they visualised the following series of reactions to occur:



(LV)

However, Isbell (1944) represented the reaction in the following manner:

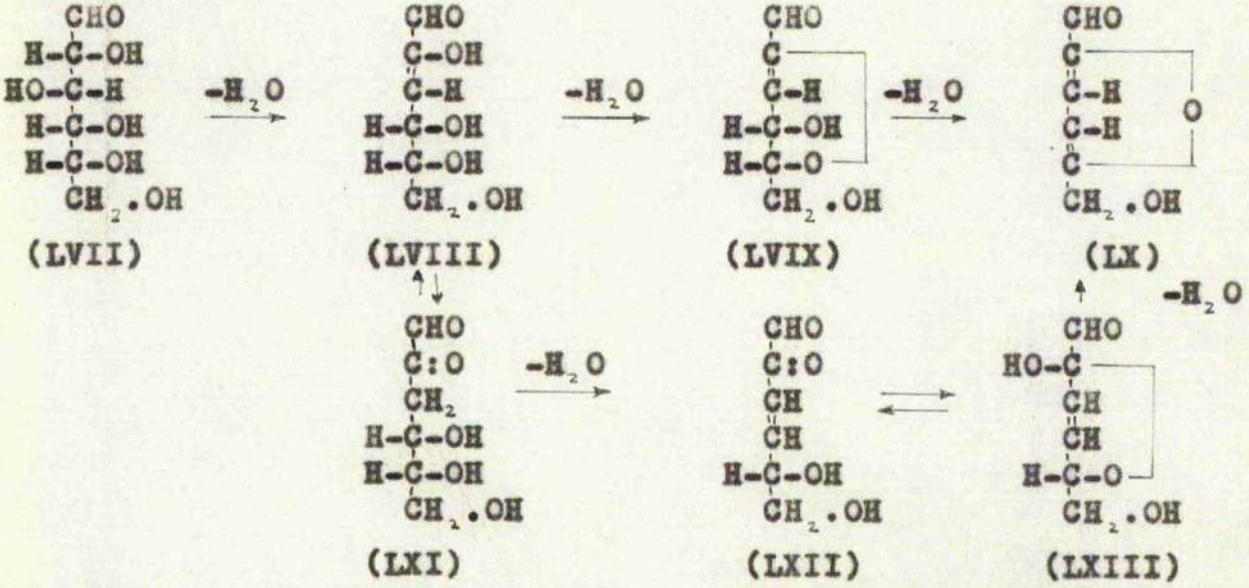


(LVI) (LV)

This mechanism, which is similar to that proposed for the conversion of tetra-O-methyl-2-hydroxyglucal to 5-methoxymethylfurfural, includes as an intermediate a 3:4-unsaturated osone (LVI) analogous to (LIV).

From a study of the change in absorption spectrum during the course of the reaction, Wolfrom, Schuetz & Cavalieri (1948) postulated a reaction mechanism for the formation of 5-hydroxymethylfurfural from glucose when aqueous solutions of the latter were heated in the absence and presence of hydrochloric acid. They proposed that an enol of 3-deoxy glucosone (LVIII) was formed by loss of the elements of water at C_2 and C_3 in glucose, reacting in the open chain form (LVII), and in turn gave rise to (LVIX) by cyclic dehydration from which 5-

hydroxymethylfurfural (LX) was formed by a final dehydration to produce a second double bond in conjugation with that already present.

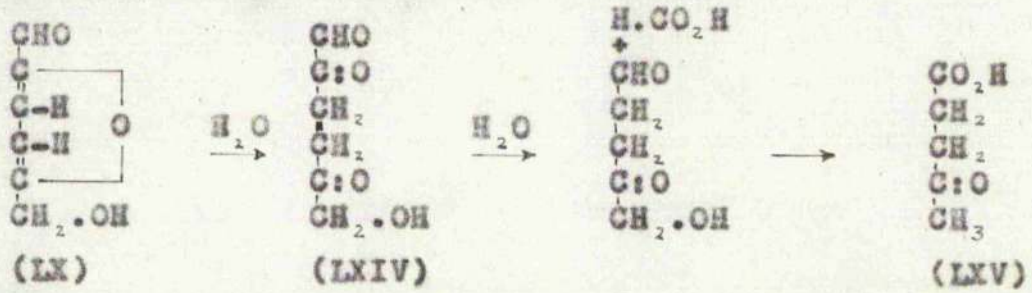


The alternative route through the intermediates (LXI), (LXII), and (LXIII) was based on an analogy with the transformation of tetra-O-methyl-2-hydroxyglucal into 5-methoxymethylfurfural. (LXIII) represents the 2:5-furanose ring form of the open chain unsaturated osone (LXII) c.f. (LIV). A similar scheme has been put forward to explain the conversion of pentoses into furfural (Wolfrom, Schuetz & Cavalieri, 1949). A route corresponding to (LVII) → (LVIII) → (LVIX) → (LX) for the formation of furfural from pentoses had previously been postulated by Hurd & Isenhour (1932) without any experimental evidence.

The disadvantage of the scheme due to Wolfrom et al. (1948) is that it applies only to glucose and does not permit an interpretation with respect to fructose. Haworth & Jones (1944) proposed a mechanism for both aldo- and keto-hexoses in which a 1:2-enediol was a common intermediate; osones were not considered to be intermediates. Such a scheme receives support from the fact that Fischer (1889) reported that glucosone under the action of dilute hydrochloric acid gave furfural and not 5-hydroxymethylfurfural (see Part I, 2.2.).

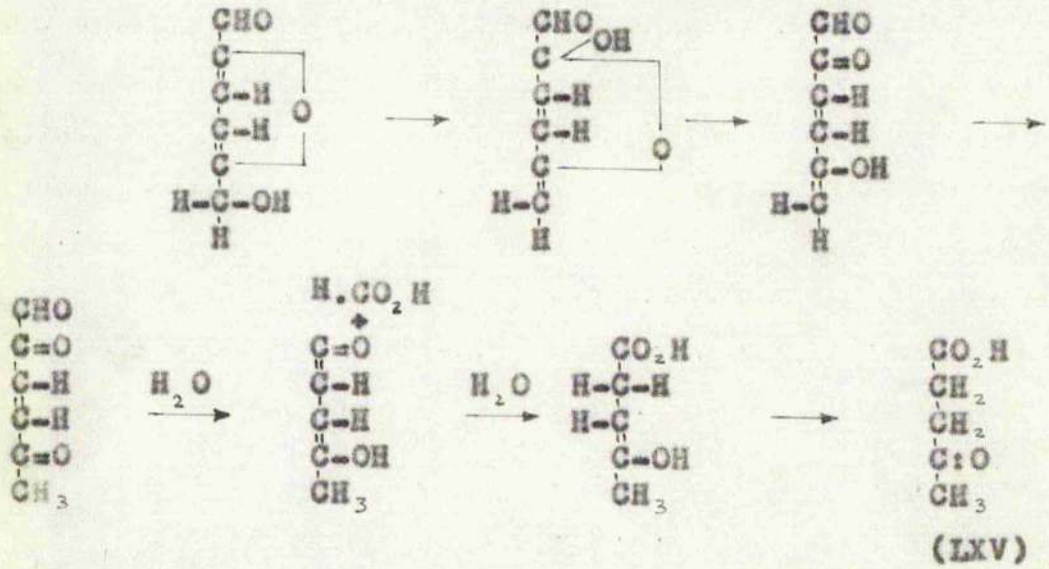
Further heating of 5-hydroxymethylfurfural (LX) produces

laevulinic acid (LXV). According to Pummerer, Guyot & Birkofer (1935) the reaction involves the opening of the furan ring with the formation of 2:5-dioxo-6-hydroxycaproic aldehyde (LXIV), which may be regarded as acyclic 3:4-dideoxy-5-oxo-glucosone, followed by elimination of formic acid and "oxidation of the aldehyde group by the primary alcohol group"; such a scheme represents a simplification of that originally proposed by Tsunissen (1930).



(LX) (LXIV) (LXV)

Isbell (1944) interpreted the reaction as follows, unsaturated osones again being suggested as intermediates:



(LXV)

Fischer (1889) reported the formation of laevulinic acid by the action of hot dilute hydrochloric acid on glucosone; similar treatment of 2-deoxy pentoses also yields laevulinic acid (Levene & Mori, 1929), a reaction which has been interpreted by Isbell (1944). Sowden (1949), by the use of glucose labelled with isotopic ^{14}C at the reducing group, confirmed that it is in fact C_1 in the glucose molecule which is finally embodied in the

formic acid produced by the decomposition of 5-hydroxymethyl-furfural.

1.6. The Biological Formation of D-Glucosone.

Walker (1932) and, later, Bond, Knight & Walker (1937) described the formation of D-glucosone by the oxidation of various sugars with plasmolysed preparations of two moulds (*A. paraciticus* Speare, and an unnamed mould belonging to the *flavus* section of the *flavus-oryzae* series of the Aspergilli). A yield of 8.6% of glucosone (identified and estimated by conversion into glucose phenylosazone) was obtained from glucose and 17% from maltose; starch and sucrose gave 15% and 13.6% yields, respectively.

Berkeley (1933) isolated an enzyme system from the crystalline style of a mollusc, Saxidomus giganteus, which, he claimed, oxidised glucose to glucosone, characterised as glucose *m*-nitrophenylosazone.

Neither of these modes of formation of glucosone has been confirmed and, in both cases, evidence for the production of an osone is incomplete, based as it is on the preparation of non-definitive derivatives. These results are more fully discussed in Part I, 5.

2. THE PROPERTIES AND REACTIONS OF THE OSONES.

2.1. Physical Properties.

2.1.1. Physical Form.

No osone has been obtained crystalline. The majority of workers have described their osone products as being syrups although Petuely (1952) has stated that, "None of the known osones of the pentoses and hexoses had so far been shown capable of being preserved when crystallised", thus suggesting that these compounds had been crystallised; however, he presented no evidence of such a fact. Petuely concluded that "it was an obvious assumption that they (the osones) were not simple bodies but mixtures of isomers or of stereoisomers". Such an assumption is far from obvious if one considers the large number of compounds in the sugar group which have been obtained crystalline only many years after their initial preparation; the existence of a sugar in the syrupy state is not inconsistent with chemical purity (see Hudson, 1945, 1951). Morrell & Crofts (1899) described the formation of a white hygroscopic solid by the addition of dry ether to an ethanolic solution of glucosone. Bayne, Collie & Fewster (1952) reported that glucosone could be obtained as "a very pale yellow, thick syrup" by evaporation of a solution of the sugar in 96% ethanol.

2.1.2. Molecular Formulae.

From its mode of formation and its reactions Fischer (1888) presumed glucosone to be an α-ketoaldehyde of molecular formula $C_6H_{10}O_6$ but did not confirm this by elementary analysis of his product. The only analysis for carbon and hydrogen carried out on a free osone was that by Morrell & Crofts (1902) for glucosone on the product of ether precipitation from ethanol; they were unable to decide between a molecular formula of $C_6H_{10}O_6$ and one of $C_6H_{12}O_6$. No molecular weight determinations have been made on osones.

2.1.3. Solubility Properties.

The unsubstituted osones have been shown to be freely soluble in water, less soluble in methanol and sparingly soluble in ethanol; the osones are insoluble in ether, petroleum ether, chloroform and acetone.

2.1.4. Optical Properties.

Fischer (1888) reported an aqueous solution of D-glucosone to be feebly laevorotatory, an observation confirmed by Morrell & Crofts (1902). Hynd (1927a) recorded an average specific rotation of -3.23° for D-glucosone in water. Micheel, Kraft & Lohmann (1934) gave a specific rotation of -6.35° for L-gulosone in aqueous solution. Becker & May (1949) noted that rotational changes took place in an aqueous solution of D-glucosone and reported a change of specific rotation from -2.55° to -1.40° (equilibrium) after 50 minutes. It should be pointed out, however, that their sample of glucosone, prepared by the method of Brüll (1936) and purified by precipitation from alkaline solution with lead hydroxide, contained in all probability, inorganic contaminants. In addition these workers examined a 0.55% aqueous solution of the osone in a 4 dm. tube and their results therefore represent the observation of a change in angular rotation from -0.056° to -0.031°; thus the significance of such observations is doubtful.

Independently, the present author (c.f. Bayne, Collie & Fewster, 1952, and Part II, 2.1.) has shown that an 8.5% aqueous solution of D-glucosone exhibits mutarotation and has observed a change in specific rotation from -10.6° to +7.9° (equilibrium) over a period of 275 hours, the initial and final rotations differing considerably from those reported by Becker & May.

Becker & Day (1953) reported a specific rotation of -1.5° for a 12.62% aqueous solution of [1-^{14}C]-D-glucosone.

2.1.5. Spectrophotometric Analysis.

Bednarczyk & Marchlewski (1938) made a study of the ultraviolet absorption spectrum of aqueous solutions of D-glucosone, prepared by Fischer's original method, and claimed to observe a selective absorption band corresponding to a free carbonyl group and similar to that exhibited by aqueous solutions of fructose and sorbose (Bednarczyk & Marchlewski, 1937, 1938). From other evidence it is known that the free keto forms of fructose and sorbose are in fact present in aqueous solution to a limited extent, but these workers admitted that their results on glucosone were only approximate owing to the possible lack of purity of their material. This work has not been confirmed by other workers in the field.

2.1.6. Chromatographic Analysis.

The first report of the application of this technique to the osones is that due to Petuely (1952). He considered that osones exist in two types of isomeric form, present in dynamic equilibrium in aqueous solution (see Part I, 4.), and claimed to confirm this assumption, based on enolisation experiments (see Part I, 2.2.4.), by paper chromatographic analysis. Osones, prepared by the benzaldehyde method, were chromatographed on paper using the upper layer of a n-butanol-acetic acid-water (4:1:5) mixture as developing solvent and a 1% solution of 3:4-dinitrobenzoic acid in 2N-sodium carbonate as spray reagent. It was claimed that glucosone, and other osones, did not produce a discrete spot but a streak which reached from an R_F value corresponding to the disaccharide lactose to that of glucose, fructose and even further. A maximum was observed at the glucose level and a weaker one at that of lactose; the latter was reported to become visible before heating and it was considered that this spot represented a form of glucosone containing one lactol ring and a free or hydrated keto group which enolised spontaneously under the influence of 2N-sodium carbonate. The spot at the glucose

level, which appeared only after heating, was considered to be a form of the osone containing two oxidic rings, which was less readily enolised. From its R_F value, Petuely suggested that the spot at the lactose level might be a dimer of the mono--lactol ring form; if this were true, it is unecessary to postulate the existence of two isomers since it has been shown that, for example, polymeric forms of pyruvic acid may be separated chromatographically (Dedonder, personal communication). Petuely made no attempt to confirm his observations employing other developers and spray reagents and presented no evidence of the degree of purity of his osone preparations; also, the proposal that the relationship between his two isomeric forms of the osones is analogous to that between the hexonolactones and the free hexonic acids is untenable since the latter equilibrium is one between two distinct structural species.

Recently, Becker & Day (1953) have described the preparation of [1-$_{14}$C]-D-glucosone by the action of pyruvic acid on [1-$_{14}$C]-D-glucose phenylosazone. They reported that the product "was homogeneous, as shown by descending paper chromatography"; no details of the composition of the developing solvent, spray reagents etc. were given nor were any R_F values recorded.

2.2. Chemical Properties and Reactions.

2.2.1. Oxidation of Osones.

2.2.1.1. Action of Copper Reagents.

Fischer (1888) reported that glucosone reduced Fehling's solution without the application of heat and compared this reactivity with that of glucose which reduces this reagent only on warming. Later, Fischer (1889) showed this reactivity to be typical of the osones in general, a fact confirmed by subsequent workers. Reduction of copper reagents at room temperature has been widely used as a method of detection of osones in sulution, although von Vargha (1936) reported that 5-O-methyl D-glucose possessed similar reactivity; however, this was considered to be due to the existence of this latter compound in the aldehyde form in aqueous solution since it also gave a colour immediately with Schiff's reagent. Dixon & Harrison (1932) and Berkeley (1933) claimed that glucosone regenerated the colour of Schiff's reagent, but such a reaction has not been observed by other workers (Brüll, 1937; Bayne, Collie & Fewster, 1952). 2-Oxo- and 5-oxo-D-gluconic acids have been reported by Militzer (1944) to reduce Benedict's reagent at room temperature. Thus, reduction of copper reagents without the application of heat is by no means a specific test for osones.

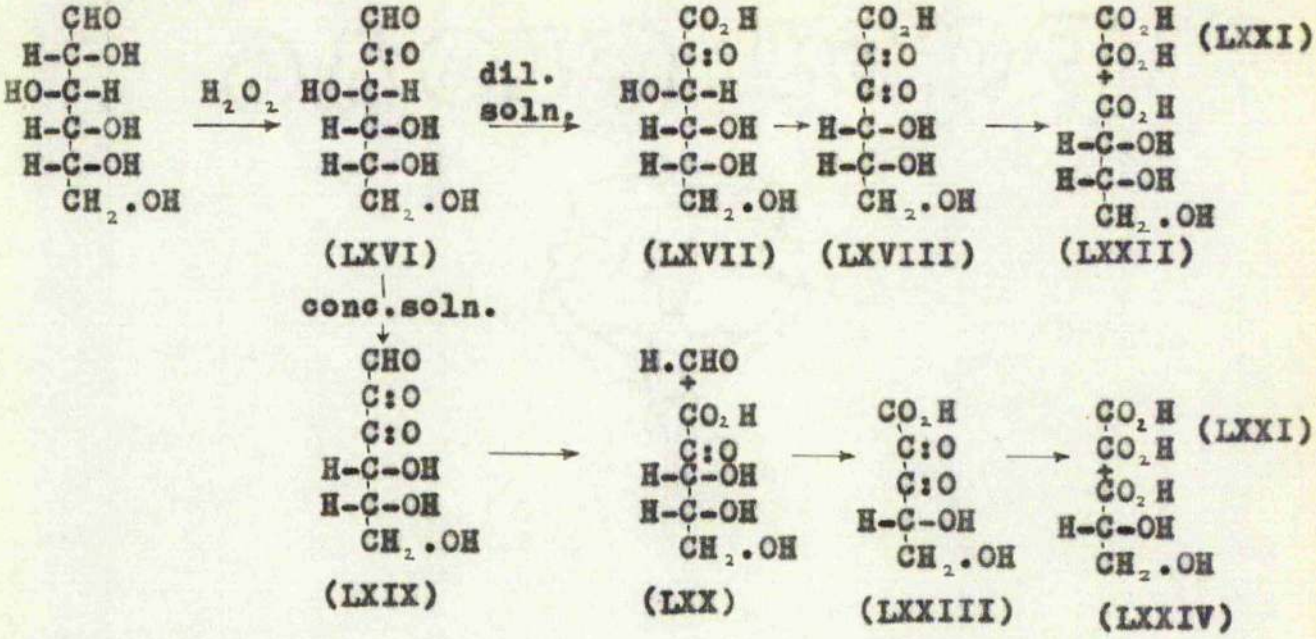
The products of further oxidation of glucosone in the presence of cupric acetate were described by Evans, Nicoll, Strause & Waring (1928); two simultaneous pathways of degradation were proposed. Oxidation of the osone was considered to produce 2-oxo-gluconic acid which then suffered decarboxylation, while enolisation of the osone formed the corresponding 2:3-enediol. By oxidative rupture of this latter enediol the "active form of erythrose" and the "half active form of glyoxal" were formed and further degraded to glyoxylic, oxalic and glycollic acids. No direct proof of the formation of enols in acid

solution was offered by these investigators.

2.2.1.2. Action of Fenton's Reagent.

Morrell & Crofts (1903b), showed that "over-oxidation" of glucose or fructose with Fenton's reagent led to the production of acids, identified as glycollic, glyoxylic, oxalic, and trihydroxybutyric acids; they considered that these acids were formed by oxidative rupture of the glucosone molecule, which was the first product of oxidation. Morrell & Crofts (1903b) also suggested that concentration of osone preparations in the presence of trace of iron caused degradation with the production of keto acids. The findings of Morrell & Crofts were confirmed by Spoehr (1910).

The nature of the products formed by the oxidation of carbohydrates by Fenton's reagent under various conditions and the mechanism of the reaction have been fully investigated by Küchlin (1932, 1933). He showed that at low temperatures and in dilute solution the following products were formed from glucose: glucosone (LXVI), 2-oxo-gluconic acid (LXVII), and 2:3-di-oxo-gluconic acid (LXVIII); in concentrated solutions, formaldehyde and 2-oxo-arabonic acid (LXX) were also formed via the di-oxo compound (LXIX). It was assumed that the primary product of oxidation was glucosone and thus the other compounds represent the products of oxidation of glucosone with Fenton's reagent. At higher temperatures, carbon dioxide, formic acid, oxalic acid, glycollic acid, tartronic acid, glyceric acid and other acids were shown to be formed. The carbon dioxide was considered to arise by decarboxylation of the 2:3-di-oxo acid (LXVIII) with the simultaneous production of 2-oxo-arabonic acid (LXX), and from this same acid oxalic (LXXI) and trihydroxybutyric (LXXII) acids were produced by cleavage of the $C_2 - C_3$ bond; further oxidation of the 2-oxo-arabonic acid (LXX) gave the corresponding 2:3-di-oxo acid (LXXIII) which on cleavage yielded oxalic (LXXI) and glyceric (LXXIV) acids.



2.2.1.3. Action of Halogens.

Morrell & Crofts (1902) reported the degradation of D-glucosone by oxidation with bromine water at 40° to give a 25-30% yield of trihydroxybutyric acid, characterised as its calcium or barium salt. Since the starting material, which was obtained in small yield, was prepared by the action of Fenton's reagent on glucose or fructose, and identified as D-glucosone solely on the grounds of osazone formation, its purity is questionable. In contrast, Neuberg & Kitasato (1927) obtained 18g. of calcium 2-oxo-D-gluconate from 20g. of D-glucosone (prepared from D-glucose phenylosazone) by the action of bromine water at 20°. By a similar method Kitasato (1929) prepared 2-oxo-D-galactonic and 2-oxo-maltobionic acids from the corres: ponding osones, characterising the products as the brucine salts because of the amorphous nature of the calcium salts.

No halogen oxidation of osones in buffered weakly acid solution has been reported but Cook (1941) patented the prepar: ation of 2-oxo-aldonic acids by the electrolytic oxidation of osones in a buffered solution consisting of calcium carbonate and some calcium bromide. This electrolytic method, first employed by Isbell & Frush (1931) for the oxidation of aldoses,

is an indirect oxidation, as the small amount of bromide present is continuously converted to bromine, the actual oxidant. Essentially, the reaction is the same as the action of bromine in weakly acidic buffered solution, when the prevention of the accumulation of hydrobromic acid in turn prevents inhibition of the oxidation.

The action of the hypohalites is generally far more drastic in alkaline solution than in acid or netural media. The effect may be attributed in part to the action of the alkali on the sugars, the oxidation not being confined to the reducing group alone. Under carefully controlled conditions, however, alkaline hypoiodite has been employed as an analytical reagent as well as for the preparation of aldonic acids (Romijn, 1897; Willstätter & Schudel, 1918; Goebel, 1927; Kline & Acree, 1930; Myrbäck, 1938). "Over-oxidation" of aldoses leads to the production of 2-oxo-aldonic acids, which subsequently split off carbon dioxide and form a lower aldonic acid (Hönig & Tempus, 1924); other workers (Reichstein & Neracher, 1935; Ruzicka, 1941) have claimed that the main products are 5-oxo aldonic acids. Under the conditions employed for the estimation of aldoses by hypoiodite oxidation ketoses are not oxidised to any great extent (Romijn, 1897).

The preparation of 2-oxo-D-gluconic acid from D-glucosone was reported by Myrbäck (1939). The osone was oxidised in alkaline solution with hypoiodite; as the oxidation velocity was the same as that for D-glucose under the same conditions it was assumed that the 2-oxo group had no particular effect on the aldehyde group. That the osone was less stable in sodium hydroxide solution than was glucose was recognised, but in an alkaline solution of sodium hydroxide and sodium bicarbonate this effect apparently was minimised. It may be deduced from these results that glucosone, under these conditions, is oxidised in the form of 2-oxo-β-D-glucopyranose. It may be suggested that

if, in alkaline solution, the 2-oxo group of D-glucosone were hydrated an oxidation velocity dissimilar to that of β-D-glucose but similar to that of β-D-mannose (Myrbäck, 1940) might be expected.

2.2.1.4. Action of Reagents Cleaving Glycols.

Fleury & Fievet-Guinard (1947) showed that glucosone was oxidised regularly by periodic acid to formaldehyde, formic acid, and glyoxylic acid which was further degraded to formic acid and carbon dioxide (see Part I, 4.).

Becker & May (1949) observed a fairly rapid utilisation of two moles of lead tetra-acetate per mole of glucosone in glacial acetic acid, followed by a slow progressive oxidation over several days, no significant amount of formaldehyde being formed (see Part I, 4.).

2.2.2. Reduction of Osones.

Fischer (1889) reduced glucosone, prepared from glucose phenylosazone, with zinc dust in aqueous acetic acid and obtained fructose; such a transformation represented the first chemical conversion of an aldose into a ketose. Reduction to fructose, identified by the Seliwanoff test, was used by Berkeley (1933) for the detection of glucosone in biological material. Percival & Percival (1935), who claimed to prepare 5-O-methyl D-glucosone and 3:4:5-tri-O-methyl D-glucosone by decomposition of partially and fully methylated D-glucose phenylosazone respectively, obtained the corresponding fructose methyl ethers by reduction of the substituted osones after the manner of Fischer (1889); Hartley & Linnell (1940) reported the preparation of 6-O-methyl D-fructose by similar reduction of 6-O-methyl D-glucosone.

2.2.3. Action of Acids on Osones.

Fischer (1889) obtained furfural characterised as furfural phenylhydrazone, by the action of hot dilute mineral acid on solutions of D-glucosone; laevulinic acid, identified as its

silver salt, was also formed.

A positive reaction with Molisch's reagent, which is considered to depend upon the formation of furfural or a derivative thereof, is given by glucosone. That glucosone does not yield furfural as readily as do ketoses is shown by the observation that glucosone does not give a positive reaction with Seliwanoff's test (Hynd, 1927a).

The formation of furfural from pentoses and of 5-hydroxymethylfurfural from hexoses by the action of dilute mineral acid has been proposed by various workers to proceed via osone derivatives (see Part I, 1.5.), as has the formation of laevulinic acid by further heating of acid solutions of 5-hydroxymethylfurfural.

Zerban & Sattler (1950) reported that glucosone gave no coloration with the anthrone reagent of Dreywood (1946) and claimed that such an observation supported their hypothesis that a positive result with this reagent was dependent upon the formation of furfural; this claim was made without consideration of Fischer's (1889) report of the formation of furfural from glucosone by the action of acid and Dreywood's (1946) observation that the coloration given by furfural with the anthrone reagent differs from that given by the sugars.

2.2.4. Action of Alkalis on Osones.

In Part I, 1.5. a number of reactions involving the degradation of sugars by alkali in which osones have been preposed as intermediates has been reviewed. It is the intention in this section to consider only the mechanism of the formation of kojic acid derivatives from osones, (see Part I, 1.4.1.), together with some recent work by Petuely (1952) on the enolisation of osones.

The conversion of tetra-O-acetyl glucosone hydrate into di-O-acetyl kojic acid was first reported by Maurer (1930); later, he and his co-workers showed tetra-O-acetyl galactosone

hydrate, as well as various benzoyl derivatives of glucosone, to undergo this transformation. It should be noted that since kojic acid contains no asymmetric carbon atoms it should be possible to obtain the compound from any hexosone.

Maurer & Petsch (1931) suggested that the transformation of the osone derivatives into the γ-pyrone structure, which involves a rearrangement of the potential carbonyl group from C_2 to C_3, took place via a common enol form with the production of an unstable "oxidation product of glutose" ("glutose" was considered to be 3-oxo-glucose), which stabilised itself as kojic acid.

Isbell (1944) has pointed out that the experimental conditions required for the transformation are similar to those which effect the transformation of penta-O-acetyl-ketoinositol to tetra-acetoxybenzene (Posternak, 1941), and the conversion of α-hydroxy ketones into diacetates of enediols (Barnes & Tulane, 1946). Isbell proposed a mechanism for the conversion of tetra-O-acetyl glucosone hydrate (LXXV) into di-O-acetyl kojic acid (LXXVI) in accordance with an electronic concept which involved the following reactions:

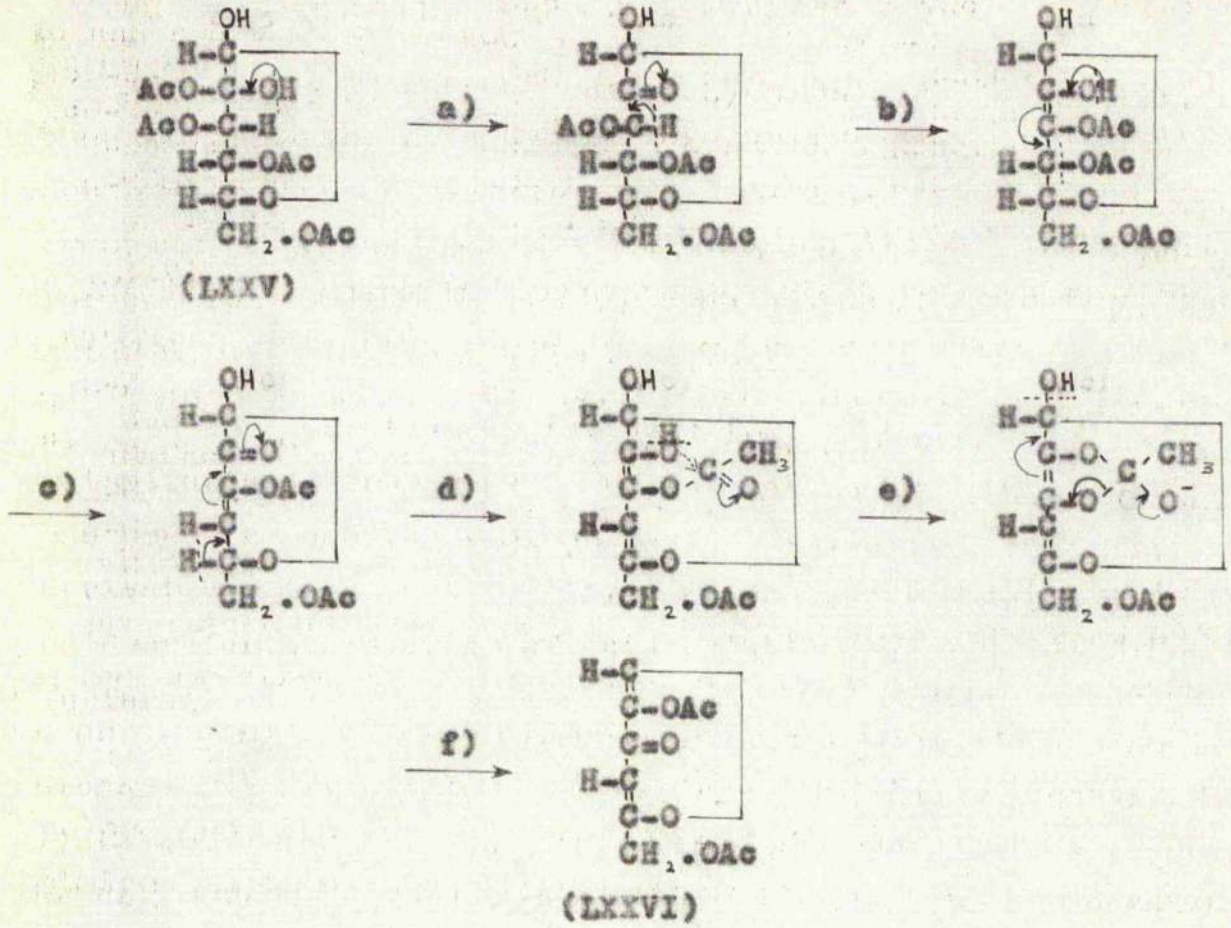
a) formation of a free carbonyl group at C_2,

b) enolisation of the carbonyl group,

c) de-enolisation of the enediol with elimination of the acetyl group at C_4,

d) enolisation of the resulting compound through the conjugated double bond,

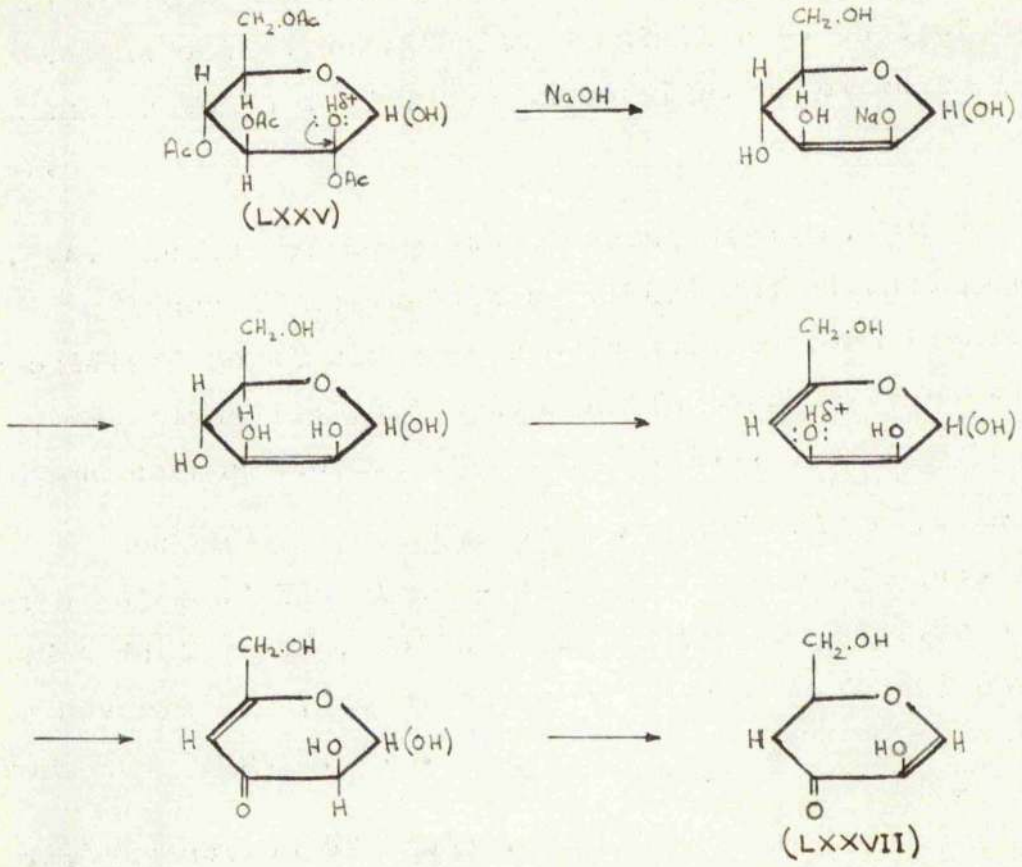
e) and f) migration of the acetyl group on C_3 to C_2 by means of an intermediate of the ortho-ester type, with elimination of a proton and an acetate ion.



(LXXV)

(LXXVI)

Stacey & Turton (1946) investigated the properties of tetra-O-acetyl glucosone hydrate and demonstrated that the compound contained an incipiently ionic hydrogen atom. With regard to the conversion into di-O-acetyl kojic acid they claimed that their experimental observations agreed in some measure with Isbell's theoretical considerations, but did not consider that some of his postulates, particularly those envisaging the formation of ortho-acetate structures, were necessary; thus, they showed that even simple alkali treatment converted the osone derivative (LXXV) into free kojic acid (LXXVII) (identified spectrophotometrically). The following mechanism was proposed for the latter transformation, the action of the alkali, besides effecting de-acetylation, causing enolisation with the resultant formation of the sodium salt of the enol



A similar mechanism was proposed for the conversion of tetra-O-acetyl glucosone hydrate into di-O-acetyl kojic acid by the action of pyridine in the presence of acetic anhydride, the pyridinium ion replacing the sodium ion; however, such a mechanism would not apply to the same transformation brought about by pyridine alone or aqueous solutions of pyridine since at one stage in the mechanism a re-acetylation was proposed which could hardly take place in such media.

Vogel (1937) demonstrated that piperidine catalysed the rearrangement of methyl 2-oxo-D-gluconate into D-glucoascorbic acid to an extraordinary degree. The action of piperidine on tetra-O-acetyl D-glucosone hydrate resulted in an increase of the reducing power which gradually decreased on prolongation of the action of the base; Vogel formulated the product of this reaction as a 2:3-dienol and designated it D-glucoascorbinal.

Petuely (1952) described an investigation of the enolisation of glucosone by 0.1N-sodium hydroxide, the degree of enolisation at various time intervals being determined by titration with Tillman's reagent (2:6-dichlorophenolindophenol). After 2 hours 32% of the osone was considered to be enolised of which 10.8% was represented by reductones of the type R.CO.C.OH : C.OH.R, the remaining 21.2% being non-reducing after acidification. From these results, Petuely proposed that in solution glucosone exists as a mixture of two types of isomer in dynamic equilibrium - one form containing a free keto group and one lactol ring, and being readily enolised; in the second, containing two lactol rings, the rate of enolisation would be limited by the rate of opening of the second lactol ring. He considered that such proposals would explain the apparent slow rate of enolisation, and claimed to confirm these results by chromatography on paper of osone solutions (see Part I,2.1.6.). Petuely presented no evidence as to the purity of his osone preparations and did not consider the possibility of the occurrence of two simultaneous reactions of a single structural form under the influence of alkali. Thus his results could be explained on the grounds of partial enolisation, together with a transformation of the osone molecule into kojic acid, a transformation which has been shown to occur on alkali treatment of tetra-O-acetyl glucosone hydrate (Stacey & Turton, 1946).

Glucosone has been shown to react with alkali cyanide, forming a product which gives an intense blue colour with Benedict's arsenophosphotungstic acid reagent for uric acid (Bayne, Collie & Fewster, 1952).

2.2.5. Nitrogenous Derivatives of Osones.

2.2.5.1. Reaction with Derivatives of Hydrazine.

Fischer (1888) reported that D-glucosone formed D-glucose phenylosazone with phenylhydrazine at room temperature. The ready formation of phenylosazones under these mild conditions

was later shown to be typical of other osones (Fischer, 1889; Fischer & Tafel, 1889; Morrell & Crofts, 1899; Fischer & Armstrong, 1902), and the reaction has been used by many workers for the non-specific detection, characterisation, and estimation of osones (see Part I, 1.2 - 1.6 and Part I, 2.2.6.). The ready formation of osazones with substituted phenylhydrazines such as methylphenylhydrazine (Fischer, 1889; Morrell & Crofts, 1899), diphenylhydrazine (Fischer, 1889), p-bromophenylhydrazine (Morrell & Crofts, 1903b), 2:4-dinitrophenylhydrazine (Dixon & Harrison, 1932; Bond, Knight & Walker, 1937) and m-nitrophenylhydrazine (Berkeley, 1933), as well as hydrazine hydrate (Morrell & Crofts, 1899) has also been widely recognised as being characteristic of the osones, although many other derivatives form osazones more readily than do the free sugars.

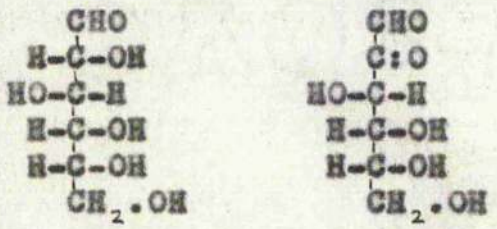
Fischer (1889) prepared a methylphenylhydrazone of D-glucosone but was unable to decide to which carbon atom of the osone molecule the substituted phenylhydrazine residue was attached; he gave no account of the properties of this hydrazone other than the melting point and the results of elementary analysis. The formation of a similar hydrazone with diphenylhydrazine was also reported. The preparation of this same methylphenylhydrazone by the present author was reported by Bayne (1953), together with a discussion of its properties (see Part II, 2.2.). Later, Ohle, Henseke & Czyzewski (1953) described the preparation of the same compound by nitrous acid degradation of fructose methylphenylosazone as well as by the oxidative action of methylphenylhydrazine on fructose; these workers claimed that it was identical with the "β-fructose methylphenylhydrazone" of Percival & Percival (1937).

2.2.5.2. Reaction with Aromatic Diamines.

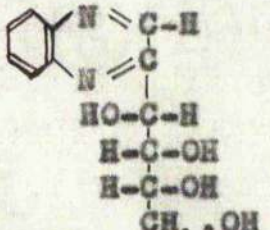
Fischer (1889) showed that the "anhydrogluco-o-diaminobenzene" prepared by Griess & Harrow (1887) by the prolonged action of o-phenylenediamine on D-glucose (LXXVIII) in the presence

of acids, was rapidly obtained from D-glucosone (LXXIX), as was the toluene homologue; Fischer pointed out that the reaction of the o-diamine with glucose was similar to the formation of glucose phenylosazone, initial oxidation providing a dicarbonyl compound which reacted in the known manner with the aromatic diamines. Later, Ohle (1934) observed that D-fructose and o-phenylenediamine also condensed to yield the same product.

The compound thus obtained is now known as 2-(D-*arabo*-tetrahydroxybutyl)-quinoxaline (LXXX)



(LXXVIII) (LXXIX)



(LXXX)

The ready formation of compounds of this type has been used for the detection and identification of osones (Fischer, 1889; Fischer & Tafel, 1889; Morrell & Crofts, 1899; Bond, Knight & Walker, 1937; Ohle, 1934; Ohle & Hielscher, 1941).

2.2.5.3. Reaction with Cyanides.

That the prediction of Hynd (1927a) that "further study might prove glucosone to be of considerable importance ..... as a starting material for the synthesis of certain carbohydrate complexes" has been fully justified is realised from consideration of the synthesis of L-ascorbic acid and its analogues by the cyanhydrin method. The preparation of osones for this type of synthesis has been already considered (Part I, 1.2.3.), while the mechanism of the reaction has been very adequately reviewed in the literature (Smith, 1946).

2.2.6. Methods of Estimation of Osones.

Ready formation of D-glucose phenylosazone has been used as the basis for the gravimetric estimation of glucosone solutions (Hynd, 1927a; Evans, Nicoll, Strause & Waring, 1928;

Bond, Knight & Walker, 1937; Weidenhagen, 1937), as has the formation of the less soluble 2:4-dinitrophenylosazone, employing the reagent of reagent of Case & Cook (1931), by Dixon & Harrison (1932) and Mandl (1950). Complete oxidation with alkaline potassium permanganate solution was used for the estimation of glucosone by Hynd (1927a), whose results obtained by this method agreed closely with those using phenylhydrazine. Saloman, Burns & King (1952), assuming that conversion of L-xylosone into imino-L-ascorbic acid by the action of alkali cyanide took place quantitatively, estimated solutions of the osone by determination of the imino-L-ascorbic acid by titration with 2:6-dichlorophenolindophenol solution; a similar method, based on determination of D-ascorbic acid formed by acid hydrolysis of the corresponding imino derivative, was employed by Hamilton & Smith (1952) for the estimation of D-xylosone.

These attempts to determine osones quantitatively have been hampered by the lack of osone standards or crystalline derivatives from which the osones might be readily prepared in pure form. In all these estimations it has been assumed that that the osones are correctly represented by the molecular formula $C_nH_{2n-2}O_n$.

3. DERIVATIVES OF OSONES.

A variety of hydrazones, phenylosazones, and condensation compounds with aromatic diamines have been prepared from osones (see Part I, 2.2.5.) and have been employed for their characterisation.

Crystalline acetates, benzoates and "glucosonides" of osones were obtained indirectly by Maurer *et al.* (Part I, 1.4.); Ohle *et al.* claimed to prepare, again indirectly, 1-*C*-methyl and 1-*C*-phenyl D-glucosone (Part I, 1.4.). None of these compounds have been obtained directly from osones.

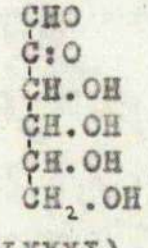
Irvine & Macdonald (1915) reported the preparation of tri-*O*-methyl D-glucosone by acid hydrolysis of 1:2-*O*-*iso*propylidene-3:5:6-tri-*O*-methyl D-glucose; the compound obtained was later (Irvine & Patterson, 1922) shown, in fact, to be 3:5:6-tri-*O*-methyl D-glucose. A number of partially methylated derivatives of D-glucosone have been prepared by decomposition of the corresponding methylated phenylosazones (see Part I, 1.2.4.). von Lebedev (1910) obtained D-glucosone-6-phosphate, isolated as an amorphous lead salt, by the action of hydrochloric acid on the phenylosazone of fructose-6-phosphate. However, no alkyl ether or phosphate ester has been prepared directly from any osone.

The only reports of the direct preparation of crystalline derivatives, from which the osones may be readily regenerated, are those of Fischer (1889), on the formation of a methylphenylhydrazone of D-glucosone, and Bayne, Collie & Fewster (1952) on the preparation of *iso*propylidene derivatives of osones; these latter compounds are fully discussed in Part II, 3.

4. THE STRUCTURE OF OSONES.

The structural features of the osones have not been definitely established; speculation concerning this problem has been considerable but all investigations have been handicapped by the lack of methods of preparation of pure osones and definitive derivatives.

From its mode of formation, its reactivity towards hydrazines, aromatic diamines, and oxidising agents, and its reduction to fructose, Fischer (1888, 1889) formulated glucosone as an α-keto aldehyde, (LXXXI).



(LXXXI)

Morrell & Crofts (1902), from the results of elemantary analysis on glucosone prepared by the oxidation of glucose or fructose with Fenton's reagent and purified by precipitation from ethanolic solution with ether, were unable to decide between $C_6H_{10}O_6$ and $C_6H_{12}O_6$ for the molecular formula of the osone.

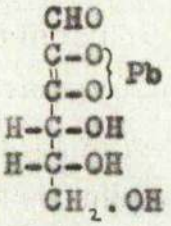
In support of Fischer's proposed structure Angelli & Marchetti (1908) reported that glucosone gave a positive hydroxamic acid reaction. Dixon & Harrison (1932) reported that glucosone, prepared by oxidation of fructose with selenious acid, regenerated the colour of Schiff's reagent and formed an addition compound with sodium bisulphite, and concluded that the molecule contained a free aldehyde group; these observations have not been confirmed by other workers and the method of preparation employed by Dixon & Harrison was not sufficiently specific to exclude the possibility that impurities may have accounted for these results. Bednarczyk & Marchlewski (1938) claimed to demonstrate a selective absorption of ultraviolet light by aqueous solutions of glucosone similar to that exhibited by

solutions of fructose and sorbose, sugars which are known to exist, to a limited extent, in a free keto form in aqueous solution. They concluded that at least a proportion of the osone in solution existed in a form containing a free carbonyl group. They admitted, however, that their results were only approximate since "it is so far impossible to get glucosone in a pure state"; their osone sample was prepared according to the "original method of E. Fischer in the form of a very nearly white substance". No indication was given by these workers of the pH of the solutions examined and their results have not been confirmed . It is interesting to recall that Niederhoff (1929) reported that ultraviolet absorption spectra of solutions of 2-oxo-gluconic acid and its salts indicated the absence of carbonyl groups, thus favouring the proposal of Ohle & Berend (1927) that the acid contained a lactol ring originating on C_2; for alkaline solutions of the acid the absorption was considered to be typical of an ethylenic or enolic linkage. Bayne, Collie & Fewster (1952) have reported that they were unable to prepare any semicarbazone or oxime from glucosone and that the sugar did not react with dimedone (5:5-di-C-methyl dihydroresorcinol) in aqueous solution; such results indicate the absence of either a free aldehyde or free ketone group in the molecule.

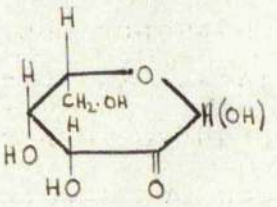
Hynd (1927a) retained Fischer's open chain formula for glucosone and pointed out that the compound "is, at one and the same time, the keto-derivative of glucose and the aldo-derivative of fructose"; however, he considered it possible that the compound might later be proved to contain one, or perhaps two, oxidic rings.

Since, in his opinion, the osones showed none of the characteristic reactions of aldehydes but did show a complete analogy with the reductones, Brüll (1937) proposed that they possessed the enediol grouping characteristic of the latter class of compounds. Previously, Evans, Nicoll, Strause & Waring (1928) had speculated that the insoluble complex formed between

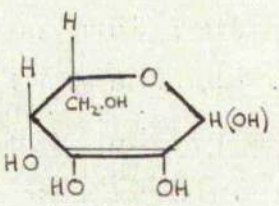
glucosone and lead hydroxide in alkaline solution (Fischer, 1889) might be the salt of a 2:3-enediol (LXXXII). Brüll considered that the osones did not have the open chain structure (LXXXI) but would exist rather in the hemiacetal form (LXXXIII) in equilibrium with the tautomeric enol form (LXXXIV), a proposal supported by Pigman & Goepp (1948).



(LXXXII)

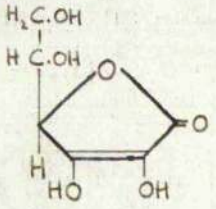


(LXXXIII)

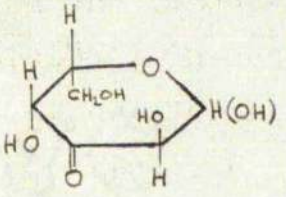


(LXXXIV)

In the examples shown the enol form (LXXXIV) is that of L-galactosone and represents, according to Brüll, a reduction product of L-ascorbic acid (LXXXV). It should also be noted that (LXXXIV) is also the tautomer of the 3-oxo derivative (LXXXVI), the glucose isomer of which Maurer & Petsch (1931) postulated as an intermediate in the conversion of tetra-O-acetyl glucosone hydrate into di-O-acetyl kojic acid by the action of pyridine.



(LXXXV)

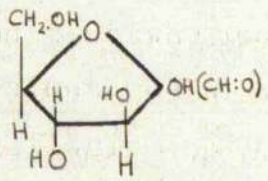


(LXXXVI)

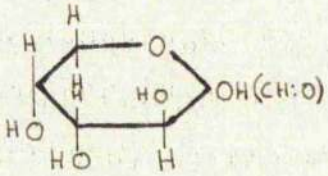
However, from Brüll's reasoning it follows that, for example, L-glucosone and L-allosone should possess a common enol form present in solution and yet each of these osones has been shown to form, by way of the cyanhydrin synthesis, a separate and distinct analogue of ascorbic acid (Haworth, Hirst & Jones, 1937; Steiger, 1935). In addition to this theoretical evidence against Brüll's hypothesis, Bayne (personal communication) and Petuely (1952) have shown that osones, in either neutral or acid media, do not decolorise 2:6-dichlorophenolindophenol solution, a result not compatible with the presence of an enediol

structure of the type present in ascorbic acid. Petuely (1952) has also demonstrated that osones are not polarographically capable of oxidation; he is of the opinion that the enolisation represented by Brüll would only occur under the influence of alkali and that the enediol (LXXXIV) would be completely unstable. Finally, it should be pointed out that Brüll's analogy between the osones and the reductones was based largely on a study of the properties of glycerosone (hydroxymethylglyoxal), a compound recognised as existing in an open chain form. Brüll's hypothesis may be rejected.

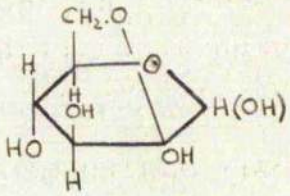
Assuming glucosone to react in the open chain form (LXXXI) Fleury & Fievet-Guinard (1947) were able to reconcile the products of periodate oxidation (see Part I, 2.2.1.4.) with such a structure; they considered that a characteristic product of such oxidation was glyoxylic acid, formed from C_1 and C_2, which was further degraded to formic acid and carbon dioxide. The first positive evidence that glucosone did contain at least one oxidic ring was supplied by Becker & May (1949) who studied the selective oxidative action of lead tetra-acetate on the compound (see Part I, 2.2.1.4.). They reported a fairly rapid initial utilisation of two moles of oxidant per mole of glucosone; no significant amount of formaldehyde was formed, from which it was presumed that the hydroxyl group on C_5 was involved in a ring structure. They also reported that an aqueous solution of glucosone exhibited mutarotation (see Part I, 2.1.4.), an observation made independently by the present author (c.f. Bayne, Collie & Fewster, 1952 and Part II, 2.1.4.). Becker & May (1949) suggested ring structures (LXXXVII), (LXXXVIII), and (LXXXIX) possible for glucosone but were unable to differentiate between them on the available evidence - formulae on p. 70.



(LXXXVII)



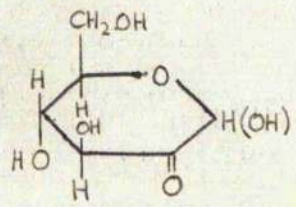
(LXXXVIII)



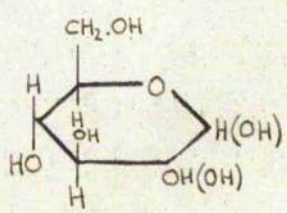
(LXXXIX)

Ohle (1931) has discussed the possible structures which may be assigned to the hexosones and has pointed out that a possible[20]/are available to each osone, each structure satisfying a molecular formula $C_6H_{10}O_6$.

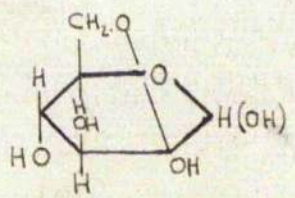
Petuely (1952) has reviewed some of the literature concerning the structure of the osones. He assumed that since "none of the known osones.....had so far been shown capable of being preserved when crystallised", they were not simple compounds but mixtures of isomers or stereoisomers; also, that a possible twenty one structures satisfying the molecular formula $C_6H_{10}O_6$ together with eight further isomers containing a hydrated keto group (either at C_1 or at C_2)could exist in an aqueous solution of glucosone. It was claimed that such assumptions were confirmed by enolisation experiments with the osone (see Part I, 2.2.4.). Petuely proposed that two species of isomer existed in aqueous solution - that containing one lactol ring (pyranose and furanose, α- and β-) and one keto group, either free or hydrated, and that containing two lactol rings (pyranose and furanose, α- and β-) in the molecule, e.g. (XC) or (XCI), and (XCII)



(XC)



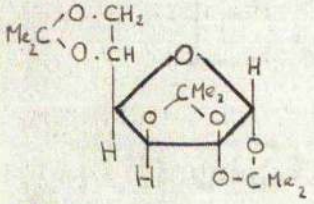
(XCI)



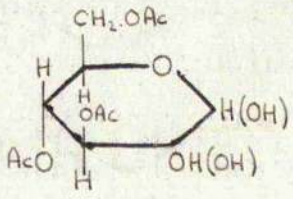
(XCII)

Petuely further claimed to demonstrate by means of paper chromatographic analysis of osone solutions (see Part I, 2.1.6.) that the two types of isomer stood in dynamic equilibrium one to another, since two "spots" were obtained. Lack of evidence of the purity of his osone preparations together with the fact that no report of the chromatographic separation of the components of a dynamic equilibrium such as that which exists between the ring form and the free keto form of fructose has been made makes acceptance of Petuely's proposals difficult.

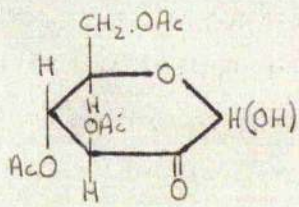
The suggestion by Petuely (1952) that glucosone could exist in a form containing one oxidic ring and a hydrated keto group at C had been preceded by the demonstration by the present author of the formation, from glucosone, of a fully substituted derivative of such a structure, namely, 1:2-2:3-5:6-tri-O--isopropylidene glucosone hydrate (XCIII) - c.f. Bayne, Collie & Fewster (1952) and Part II, 3. Also Maurer et al. had prepared acetyl and benzoyl derivatives of such a structure by methods of indirect synthesis (see Part I, 1.4.1.). Maurer & Petsch (1931) had also suggested that tri-O-acetyl glucosone hydrate (XII) and tri-O-acetyl glucosone (XIII) might exist in equilibrium in solution, although the lack of mutarotation of tri-O-acetyl glucosone reported by them does not suggest that the compound is reversibly hydrated in aqueous ethanol.



(XCIII)



(XII)



(XIII)

The results of lad tetra-acetate oxidation (Becker & May, 1949) are in agreement with a structure containing a 1:5-pyranose ring and a hydrated keto group at C_2; a structure containing a free keto group would consume only one mole of oxidant per mole in absolute glacial acetic acid (Baer, 1940) and not three moles as was observed by Becker & May (1949).

The present position with regard to the structure of glucosone may be summarised as follows: evidence points to a molecular formula $C_6H_{10}O_6$; the weight of evidence is against the existence of more than a small percentage of the molecule being present in solution in a form containing a free carbonyl group, which, together with the observation that the osone exhibits mutarotation , indicates the presence of at least one lactol ring in the molecule; the osone is capable of existing in a form containing a 1:4-furanose ring and a hydrated keto group at C_2 since on acetonation tri-O-isopropylidene glucosone hydrate is formed; various workers have proposed that glucosone, in solution, is present in several forms which are in equilibrium.

---

5. THE BIOLOGICAL SIGNIFICANCE OF OSONES.

5.1. The Biological Activity of Osones.

Fischer (1889) observed that D-glucosone was not fermented by brewer's yeast, a fact used by Morrell & Crofts (1899) to free their glucosone preparations of fermentable sugar contaminants. Fischer & Armstrong (1902) were able to hydrolyse maltosone with an aqueous extract of brewer's yeast to yield glucosone and glucose and also melibiosone with emulsin to give glucosone and galactose; Fischer & Zemplèn (1909) hydrolysed cellobiosone with emulsin.

Mitchell & Bayne (1952) were able to show that D-glucosone, as prepared by the present author, did exert an effect on yeast cells actively fermenting D-glucose. D-Glucose (0.025M) and D-glucosone (0.05-0.20M) were incubated with a suspension of baker's yeast, in the presence of cyanide as inhibitor of respiration, in the Warburg apparatus. No inhibition of glucose fermentation occurred at the lowest concentration of glucosone, while complete inhibition was observed at the highest concentration. Preliminary indications suggested that the inhibition was competitive. L-Glucosone, prepared and purified by the present author by a procedure identical with that used for D-glucosone, and tested under the same conditions as the D-isomer, showed no inhibitory effect at any concentration up to 0.20M. In a later communication it has been reported that D-glucosone is phosphorylated by yeast hexokinase (Johnstone & Mitchell, 1953).

Levene & Meyer (1915), using material prepared by the original method of Fischer (1889), were the first workers to test D-glucosone physiologically. Structurally they regarded the osone as a substituted glyoxal which might be converted to D-gluconic acid by the action of tissue enzymes, e.g. glyoxalase. However, they could detect no chemical change when glucosone was incubated with kidney tissue and concluded that the kidney enzymes acted only on the intact hexose.

Examining the action of glyoxalase on compounds of the general type R.CO.CHO Efendi & Ryzhova (1939) incubated aqueous extracts of rabbit liver in phosphate buffer (pH 6) with varying amounts of D-glucosone. By removal of unchanged osone as glucose 2:4-dinitrophenylosazone these workers showed that in concentrations of 0.5-1.0mg. of osone/ml. of buffered extract there was a slow decrease in concentration of osone, with a production of gluconic and mannonic acids. They also showed that the reaction, which was much slower than a control carried out on methylglyoxal, was accelerated by addition of glutathione as a specific coenzyme.

Antoniani (1935) found that D-glucosone was unchanged by the action of the enzymes present in germinating seeds, either in the presence or in the absence of glutathione.

Thannhauser & Jenke (1926) demonstrated that D-glucosone, prepared by oxidation of fructose with Fenton's reagent, was utilised by diabetics. They regarded glucosone as an intermediate, which was more readily oxidised than glucose, and concluded that diabetes mellitus resulted from the inability to form this intermediary product. In the following year Hynd (1927a) showed that D-glucosone was possessed of a remarkably specific physiological activity. To ensure that his material was free of other sugar moieties Hynd prepared D-glucosone by Fischer's (1889) method, the syrup being freed from nitrogenous and inorganic impurities at a late stage in the preparative procedure. For the purposes of control experiments, samples of D-glucosone, lactosone, and maltosone were prepared by the benzaldehyde method of Fischer & Armstrong (1902). Using either of these methods Hynd claimed a high degree of purity for the osone products. He found that subcutaneous injection of D-glucosone into a mouse suffering from insulin hypoglycaemia, far from alleviating the condition, as do glucose and fructose, increased its severity. Injection into normal mice produced definite symptons and the animals presented a condition very similar to that produced by an

injection of insulin. The minimum lethal dose was approximately 2.6mg./g. body weight, the animals dying in coma. Lactosone and maltosone had no physiological action even if prepared by a method similar to that employed for the preparation of D-glucosone. On the other hand the physiological effects of the products of acid hydrolysis of either lactosone or maltosone were qualitatively identical with those produced by D-glucosone prepared directly. Hynd concluded that the "osone effect" was specific for D-glucosone and was not due to traces of impurity formed during the preparation. He showed that injection of glucose after the full development of the symptoms due to a dose of glucosone had no modifying action; previous or simultaneous injection of glucose diminished the glucosone symptoms. Similarly, the development of these symptoms was modified or inhibited by either adrenalin or pituitrin. A possible significance of glucosone in fat metabolism was suggested by the observation that injection of acetoacetic acid antagonised the effect of glucosone injection.

Hynd (1927b) reported that, in the case of mice and rats, a subcutaneous injection of glucose, or of glucose plus insulin, afforded no protection against the toxic action following (a) the subsequent injection of either alkali or methyl cyanides, or (b) the inhalation of gaseous hydrogen cyanide. Under the same experimental conditions, glucosone appeared to exert a definite antagonistic action towards cyanides, but was less efficient than cystine. The previous, or simultaneous, administration of glucosone or of glucose plus insulin, appeared to be protective against the intravenous injection of alkali cyanide into rabbits, but the results obtained were somewhat variable.

The results of the experiments on mice (Hynd, 1927a) were confirmed by Herring & Hynd (1928) for white rats, rabbits, guinea pigs, and cats and the behavioural differences between the train of symptoms produced by insulin and that by glucosone

in the various species elaborated. It was shown that the mode of administration did not alter the nature of the glucosone symptoms but only their rapidity of develoment and intensity of effect. Herring & Hynd also confirmed the toxic effect of mehhylglyoxal, first reported by Sjollema & Seekles (1926), and observed that the symptoms it produced on injection were less like those caused by insulin than were those caused by glucosone. They pointed out that this apparently contradicted the now obselete hypothesis of Fischler (1927) that the toxic action of insulin, apart from the hypoglycaemia, was due to the production of an excess of methylglyoxal.

From the results of these two series of investigations, Hynd suggested that "glucosone is an important intermediate in carbohydrate metabolism, and that its formation results from the action of insulin on the blood glucose".

The specificity of the "D-glucosone effect" has been confirmed by Bayne (1952a) by experiments with a series of osones prepared and characterised by improved methods. No toxic effects were produced in mice by the following osones at various levels up to 10.0mg./g. body weight: D- and L-xylosone, D- and L-arabinosone, L-glucosone, D-galactosone, L-gulosone, D-altro-(D-gluco)-heptosone (sedoheptulosone), and 3-O-methyl D-glucosone. Bayne concluded from these results that the toxicity of D-glucosone was not dependent on the high chemical reactivity associated with the potential keto-aldehydic osone structure.

Kermack, Lambie & Slater (1929) claimed that the dimeric form of hydroxymethylglyoxal (hydroxypyruvic aldehyde) was highly toxic to mice and rabbits, causing "symptoms similar to those described by Herring & Hynd (1928) as being produced by glucosone". Hynd (1930, 1931) pointed out that such a result could be predicted, "as hydroxymethylglyoxal (glycerosone) bears the same relationship to the trioses that glucosone does

to glucose", and that Kermack et al. gave no explanation as to why the monomeric form of hydroxymethylglyoxal was less toxic. Hynd showed that the highly toxic effect on mice and rats of the polymer, prepared according to the method of Evans & Waring (1926), was due to the presence of an unstable contaminant possibly formed by the action of the hydrogen sulphide employed for the removal of excess copper oxidant during the preparation, and that a highly toxic product was also formed when glyoxal, but not acetic acid, glucose, fructose, or glucosone, was treated with hydrogen sulphide. Hynd (1930, 1931) was able to demonstrate that both monomeric and polymeric forms of hydroxymethylglyoxal, when prepared by a method not involving the use of hydrogen sulphide, exerted practically the same effect when injected subcutaneously into mice or rats. The train of symptoms produced in either case was quite distinct from that caused by glucosone, but closely resembled that produced by pure glyoxal in either the monomeric or polymeric state.

The comparative metabolism of [1-$_{14}$C]D-glucose and [1-$_{14}$C]D-glucosone, administered orally, in rats was investigated by Becker & Day (1953) through determinations of the amount of $_{14}$C from these compounds recovered in the exhaled carbon dioxide, liver and muscle glycogen, and serum glucosamine. Preliminary results showed that fresh glucosone solutions are absorbed from the alimentary tract of rats at about the same rate as glucose solutions of similar volume and concentration. It was found that both glucose and glucosone can be converted to glucosamine and that glucosone may be changed to glucose, both conversions apparently occurring without skeletal rearrangement. With glucosone more $_{14}$C was found in the glucosamine than in the animals given labelled glucose; Becker & Day (1953), however, pointed out that, "until it is rigorously proved that glucosone goes to glucosamine only via the route followed by glucose, it cannot be concluded that the rate of conversion of glucosone to glucosamine is greater than that of glucose. The dilution of

^{14}C via different pathways might not be the same. A double labeling of the glucosone would be required to prove more rigorously that there was no cleavage in the conversion. The data presented here do not demonstrate that glucosone is a normal intermediate but they are in accord with the suggestion that its formation may be a step in glucosamine synthesis".

5.2. The Biological Formation of Osones.

Dixon & Harrison (1932) were unable to detect D-glucosone (by the formation of D-glucose 2:4-dinitrophenylosazone) in the blood of rabbits in hypoglycaemic convulsions following a large over-dose of insulin, or after incubating fructose with liver tissue in the presence of insulin. In a harsh criticism of Hynd's deductions these workers ignored much of his subsidiary evidence, overlooked the specificity of D-glucosone, and took no account of the findings of Thannhauser & Jenke (1926). Dixon & Harrison (1932) concluded that D-glucosone was not an intermediary metabolite in the animal organism.

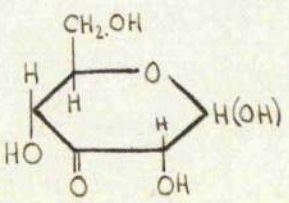
Support for Hynd's proposal that D-glucosone was a metabolic intermediate was given by the findings of Berkeley (1933), who obtained an active oxidase preparation from the crystalline style of a mollusc, Saxidomus giganteus. He showed that the oxidase system depended for its activity on at least two components, a peroxidase contained in the style itself and an autoxidisable substance in the diatoms constituting the food of the animal. Anticipating that the substrate of this system was a carbohydrate, Berkeley obtained from D-glucose, under aerobic conditions, not an acid product but D-glucosone; the osone was identified on the basis of a positive reaction with Schiff's reagent, formation of D-glucose m-nitrophenylosazone, and a reduction to fructose, identified by Seliwanoff's test. Berkeley also demonstrated the presence of a dehydrogenase system in the style, which similarly depended for its activity on the joint action of a component in the style and another in the

food material. The dehydrogenase system functioned anaerobically, converting D-glucose to D-glucosone only in the presence of a hydrogen acceptor such as methylene blue. Berkeley suggested that the oxidase system acted as the requisite acceptor *in vivo*, thus promoting the action of the dehydrogenase system. However, he obtained no direct evidence that D-glucosone was the metabolic product in the intact mollusc, and there has been no confirmation of Berkeley's work.

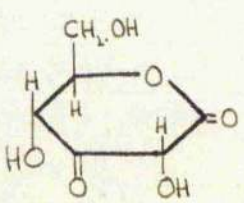
In view of a preliminary report by Walker (1932) of the formation of D-glucosone by enzymic action and the previously described publication of Berkeley (1933), Bond, Knight & Walker (1937) further investigated the production of the osone by mycological agency. Formation of D-glucosone was observed in the case of two moulds (*A. parasiticus* Speare, and an unnamed mould belonging to the *flavus* section of the *flavus-oryzae* series of the Aspergilli) when allowed to act upon soluble starch, maltose, sucrose, and D-glucose after plasmolysis by toluene, bromobenzene or chloroform. Negative or doubtful results were obtained with lactose, mannose, D-fructose, D-xylose, D-mannitol, and glycerol. The D-glucosone formed was characterised as D-glucose phenylosazone, D-glucose 2:4-dinitrophenylosazone, and 2-(D-*arabo*-tetrahydroxybutyl)-quinoxaline. Since it was found that appearance of the osone was observed only when the mould mycelium was subjected to the action of plasmolytic agents Bond *et.al.* were unable to decide whether the oxidation was due to one specific enzyme or to the activity of the residue of an enzyme complex, a component of which was destroyed by the plasmolytic agent. The production of D-glucosone by the action of plasmolysed mould mycelia on D-glucose was observed only in the cases of two species which were known to give rise to good yields of kojic acid when grown on D-glucose. Bond *et al.*, however, were unable to demonstrate that glucosone was a normal intermediary in the

conversion of glucose into kojic acid. The chemical conversion of glucose into the γ-pyrone, via tetra-O-acetyl D-glucosone hydrate, had been achieved previously by Maurer (1930) (see Part I, 1.4.1.).

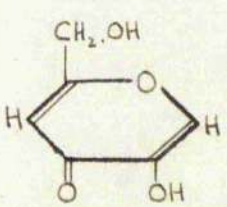
A theory that the biosynthesis of kojic acid proceeds directly from glucose without rupture of the carbon chain has in general found little support, the main objection to such a theory being the fact that kojic acid can be formed from compounds having fewer carbon atoms than glucose. It has been proposed by a number of workers (see review by Barham & Smits, 1934) that kojic acid is formed from widely different compounds by primary splitting to a common, small, reactive molecule containing two or three carbon atoms followed by condensation directly to the γ-pyrone, or a C_6 precursor of it. Recently, however, Arnstein & Bentley (1953), investigating the conversion of [1-^{14}C] and [3:4-$^{14}C_2$]-D-glucose by two species of Aspergilli, have shown that the major pathway of kojic acid formation is a direct transformation of glucose without rupture of the chain; they suggested that a minor route is the condensation of triose-phosphates. In a discussion of the mechanism of the conversion, Arnstein & Bentley suggested that one stage might be the formation of 3-oxo-D-glucose (XCIV), formed either directly by oxidation of glucose or by way of 3-oxo-D-gluconolactone (XCV); loss of the elements of two molecules of water by 3-oxo-D-glucose, or loss of a molecule of water followed by reduction and the loss of a further molecule of water by the 3-oxo-lactone would give kojic acid (LXXVII).



(XCIV)



(XCV)



(LXXVII)

3-Oxo-D-glucose was proposed by Maurer & Petsch (1931) to be an intermediate in the chemical conversion of various acetates of D-glucosone hydrate into kojic acid, but Arnstein & Bentley (1953) gave no consideration to the possibility that glucosone might be an intermediary in the mycological formation of the γ-pyrone from glucose.

For comparison it is worth summarising the results of some other workers concerning the primary products of biological oxidation of D-glucose. Conversion into D-gluconic acid was effected by the agency of an aerobic oxidase extracted from the mycelium of Aspergillus niger and of Penicillium glaucum (Müller, 1931) and also by the action of a liver dehydrogenase (Harrison, 1931). Under the action of Acetobacter suboxidans D-glucose yields 5-oxo-D-gluconic acid, by way of D-gluconic acid, (Kluyver & de Leeuw, 1924; Stubbs, Lockwood, Roe, Tabenkin & Ward, 1940). 2-Oxo-D-gluconic acid together with 6-aldehydo-D-gluconic acid (L-guluronic acid) have been obtained by the use of Bacterium gluconicum (Bernhauer & Irrgang, 1935). Bernhauer & Gorlich (1935) also detected the formation of 2-oxo-D-gluconic acid with the latter organism, as well as with some species of Acetobacter. Pseudomonas species similarly yield 2-oxo-D-gluconic acid (Lockwood, Tabenkin & Ward, 1941). D-glucosone has not been demonstrated to be an intermediate in the formation of 2-oxo-D-gluconic acid from glucose by bacterial means.

5.3. Summary and Conclusions.

It has been clearly established that D-glucosone, unlike all other osones so far tested, is possessed of striking biological activity; thus, it exerts a toxic effect on the mammalian organism and inhibits the fermentation of D-glucose by yeast. Evidence has been obtained to indicate that D-glucosone may be a normal intermediary metabolite in mammals while formation of the osone by mycological agencies has been reported. However,

the exact mode of action of D-glucosone together with its biosynthesis have not been determined. Many investigations have suffered from the lack of methods of preparation, characterisation, and estimation of pure glucosone, while final explanation of the biological behaviour is also dependent upon elucidation of the structural features of the osone.

PART II: A DISCUSSION OF THE METHODS AND RESULTS OF RESEARCH CARRIED OUT BY THE AUTHOR.

CONTENTS.

1. THE PREPARATION AND FORMATION OF OSONES.

1.1. Introduction.

1.1.1. Preparation of Some Partially Methylated Hexoses.

3-O-Methyl-1:2-5:6-di-O-isopropylidene D-glucose, prepared by methylation of di-O-isopropylidene D-glucose according to the method of Glen, Myers & Grant (1951), was hydrolysed by boiling an aqueous suspension of the derivative in the presence of cation-exchange resin to give crystalline 3-O-methyl D-glucose.

3:5:6-Tri-O-methyl D-glucose was obtained by a modification of the method outlined above employing 1:2-O-isopropylidene D-glucose as starting material.

Hydrolysis of tri-O-methyl inulin, prepared by the method of Haworth & Streight (1932), according to the directions of Haworth, Hirst & Percival (1932) gave syrupy 3:4:6-tri-O--methyl D-fructose.

1.1.2. Preparation of Osazones.

For the preparation of osones by decomposition of the corresponding osazones the initial isolation of a pure osazone is desirable. The phenylosazones of D-glucose, 3-O-methyl D-glucose, D-galactose, and D-xylose were prepared in good yield and high state of purity by employing the catalytic effect of p-toluidine observed by Weygand (1940); the N-p-tolyl-isoglycosamine (or N-p-tolyl-1-amino-1-desoxy ketose, to use the nomenclature of Barry & Honeyman, 1952), formed by fusion of the sugar with the aromatic amine followed by Amadori rearrangement in the presence of acetic acid, was not isolated in most cases but was allowed to react with a slight excess of phenylhydrazine in 2N-acetic acid. By this method phenylosazones, requiring little further purification, were obtained in 70-75% yield; reaction of pure N-p-tolyl-D-isoglucosamine (N-p-tolyl-1-amino--1-desoxy D-fructose) with phenylhydrazine gave D-glucose

phenylosazone in almost quantitative yield, as reported by Weygand (1940), but the losses involved in isolation of the intermediate made the yield of phenylosazone, calculated on D-glucose used, no more than 75%. L-Glucose phenylosazone was prepared by the action of phenylhydrazine in acetic acid on the mixture of L-glucose and L-mannose obtained by acid decomposition of L-arabinose with nitromethane (Sowden & Fischer, 1947). The phenylosazones were purified by recrystallisation from absolute ethanol, the degree of purity being assessed by observation of the final rotation after repeated recrystallisation. After one recrystallisation from absolute ethanol 3-O-methyl D-glucose phenylosazone showed a double melting point (167 and 175°) while further recrystallisation gave a product melting at 176°; such observations may explain the value of 165° given by Irvine & Scott (1913) and those values of from 174 to 179° recorded by a number of other workers (Freudenberg & Hixon, 1923; Anderson, Charlton & Haworth, 1929; von Vargha, 1934; Heddle & Percival, 1939; Weygand, 1940) for this phenylosazone. Recrystallisation of D-xylose phenylosazone from absolute ethanol gave a product melting at ~~x~~ a temperature higher than any recorded for this phenylosazone by previous workers; a similar elevation of the melting point of L-arabinose phenylosazone after such recrystallisation was reported by Haskins, Hann & Hudson (1946).

3:5:6-Tri-O-methyl D-glucose apparently does not react with p-toluidine under the conditions described above and in consequence the phenylosazone of this sugar was prepared by direct reaction with phenylhydrazine. The product obtained after recrystallisation from aqueous ethanol melted at 62°, which is considerably lower than the value reported by Anderson, Charlton & Haworth (1929); however, elementary analysis for carbon, hydrogen, nitrogen, and methoxyl groups showed the derivative to be a tri-O-methyl hexose phenylosazone. In addition it was observed that the compound exhibited

extensive mutarotation in pyridine-ethanol (2:3); no reports on the rotational behaviour of this phenylosazone appear in the literature.

For the preparation of phenylosazones from the ketoses L-sorbose and 3:4:6-tri-O-methyl D-fructose, to which the p-toluidine method is not applicable, the direct method was employed. Analytically pure 3:4:6-tri-O-methyl D-glucose phenylosazone was obtained, melting at 120°; Haworth & Learner (1928) claimed to obtain this phenylosazone as a hydrate, m.p. 87°, which lost its molecule of water of crystallisation on heating *in vacuo* to give the anhydrous form melting at 136°. Similar results were obtained by Hartley & Linnell (1939), who also prepared the phenylosazone from 3:4:6-tri-O-methyl D-fructose as well as from 3:4:6-tri-O-methyl D-glucose; it should be noted that the melting point of this phenylosazone, prepared from liquid 3:4:6-tri-O-methyl D-glucose, has been recorded by Cramer & Cox (1922) as 163-164°. This tri-O-methyl phenylosazone, for which no optical properties have hitherto been described, was also shown by the present author to exhibit striking mutarotation in pyridine-ethanol (2:3).

It was observed that the phenylosazones in which the hydroxyl group at C_3 is substituted by a methyl group, unlike those in which this hydroxyl group is free, do not decompose at the melting point, and are stable to sunlight even in the presence of atmospheric moisture. Similar stability of anhydro-phenylosazones in which the hydroxyl group at C_3 is involved in the anhydro ring structure has been observed by Bayne (personal communication).

Percival & Percival (1935) (see Percival, 1948) proposed that mutarotation of the hexose phenylosazones is dependent upon the presence of a 2:6-pyranose ring system. The observation of mutarotation of solutions of 3:4:6- and 3:5:6-tri-O-methyl D-glucose phenylosazone by the present author is not consistent with such a hypothesis; in the former methylated

phenylosazone a 1:5-glucopyranose or a 2:5-fructofuranose ring is possible, while in the 3:5:6-tri-O-methyl derivative only a 1:4-glucofuranose structure is feasible.

Neuberg & Strauss (1946) reported that the 2:4-dinitrophenylosazones of the hexoses could be obtained in almost quantitative yield in a high state of purity by carrying out the reaction with a solution of the free base in 2N-hydrochloric acid at 100° for 12-24 hours. It was considered by the present author that such osazones might provide useful starting material for the preparation of those osones of which the parent hexoses give poor yields of the corresponding phenylosazone, for example, the trimethyl ethers of glucose and fructose. D-Glucose 2:4-dinitrophenylosazone was obtained in 98% yield after a reaction time of 12 hours under the conditions described by Neuberg & Strauss (1946).

The bishydrazone of D-glucose was prepared by the action of hydrazine hydrate on N-p-tolyl-D-isoglucosamine, but was not isolated.

1.2. By Decomposition of the Corresponding Osazone.

1.2.1. Action of Hydrochloric Acid.

D-Glucosone was obtained in 30% yield by decomposition of D-glucose phenylosazone with concentrated hydrochloric acid; the method of preparation and purification followed exactly the directions of Fischer (1889). The action of the mineral acid on D-glucose 2:4-dinitrophenylosazone gave a 5% yield of D-glucosone; the greater part of the 2:4-dinitrophenylosazone was recovered unchanged. It is considered that failure of decomposition was due to the very low solubility of this osazone, even in concentrated acid.

The disadvantages of this preparative procedure have been discussed previously (see Part I, 1.2.1.). Thus, without rigorous purification of the product, involving further decrease in the yield, this method is considered to be of no value for the preparation of osones for structural investigation.

1.2.2. Action of Carbonyl Compounds.

The benzaldehyde method, originally introduced by Fischer & Armstrong (1902) for the decomposition of the phenylosazones of the disaccharides, has been exploited by a number of workers for the preparation of monosaccharide osones (see Part I, 1.2.2.); in the case of the hexosones such a mode of preparation has been characterised by low yields. However, by the introduction of various modifications, the present author has prepared a number of free and partially methylated hexosones, as well as pentosones, possessing a high degree of purity, in good yield.

The sparingly soluble phenylosazones of the unsubstituted hexoses (D- and L-glucose, D-galactose, L-sorbose), suspended in ethanol, were decomposed with a solution of benzaldehyde in hot aqueous ethanol containing 1% (v/v) acetic acid. After solution was complete a large proportion of the ethanol was removed by distillation with the concurrent addition of water, thus

precipitating the benzylidene phenylhydrazone which was filtered off. From the filtrate, after purification (see Part II, 1.5.), the osones were obtained in 50% yield. For the preparation of osones from the ethanol-soluble phenylosazones of the partially methylated hexoses (3-O-methyl D-glucose, 3:4:6-tri-O-methyl D-fructose, 3:5:6-tri-O-methyl D-glucose) and the pentoses (D-xylose) much lower concentrations of ethanol were employed. Ethanolic solutions of these phenylosazones were added dropwise to a suspension of benzaldehyde in hot 1% aqueous acetic acid. By the method of isolation outlined above the corresponding osones were obtained in 30-40% yield.

From the action of benzaldehyde on D-glucose 2:4-dinitrophenylosazone the osazone was recovered unchanged, even after 6 hours heating.

D-Glucosone was prepared in poor yield by the decomposition of D-glucose bishydrazone with benzaldehyde; severe losses were incurred during the purification of the crude product.

Brüll (1936) described the decomposition of D-glucose phenylosazone with an excess of pyruvic acid in hot aqueous solution; pyruvic acid phenylhydrazone separated on cooling and was filtered off while excess pyruvic acid was removed by extraction with ether. Evaporation of the aqueous solution, after decolorisation with charcoal, gave D-glucosone in 40% yield. The present author has obtained D-glucosone, 3-O-methyl D-glucosone, D-galactosone, and L-gulosone in 60-65% yields by a modification of the above method. The corresponding phenylosazones, suspended in water, were decomposed with a little less than the calculated amount of pyruvic acid by heating at 100° for 1-2 hours, and isolated as described above; Brüll (1936) recommended a reaction time of half an hour. By use of an excess of phenylosazone contamination of the osone products with unchanged pyruvic acid, which is very soluble in water as well as in ether, was reduced to a minimum.

By the action of glyoxal on D-glucose phenylosazone, following the procedure described for decomposition with pyruvic acid, crude D-glucosone was obtained in 70% yield by the present author. However, it was demonstrated that the product was invariably contaminated with unchanged glyoxal, in spite of employing a slight excess of phenylosazone and allowing a reaction time of 2 hours; glyoxal was not removed by extraction with ether. In consequence, such a method is of no value for the preparation of osones for structural and metabolic investigations without additional chromatographic purification or preparation of crystalline derivatives from which the osones may be regenerated (see Part II, 1.5.).

1.3. By Direct Oxidation of the Corresponding Aldose or Ketose.

1.3.1. Action of Cupric Acetate.

D-Fructose was oxidised with a saturated aqueous solution of cupric acetate at 50° for 30 hours according to the directions of Evans, Nicoll, Strause & Waring (1928). After filtration from the precipitated cuprous oxide removal of inorganic contaminants was effected by treatment with hydrogen sulphide followed by passage of the solution through columns of ion-exchange resins. The resulting solution gave qualitative tests for both osone and unchanged fructose; in consequence, the stiff syrup obtained by evaporation under reduced pressure was treated with dry acetone containing concentrated sulphuric acid (4%, by volume); the syrupy products were isolated in the usual manner and extracted with hot water. From the syrupy residue tri-O--isopropylidene D-glucosone hydrate was obtained by crystallisation from methanol (see Part II, 3.2.2.); from the aqueous extract 2:3-4:5-di-O-isopropylidene D-fructose separated on cooling. From 18g. of D-fructose 0.35g. of the isopropylidene D-glucosone derivative and 8.5g. of di-O-isopropylidene D-fructose were obtained. Calculated on the basis of a 15% conversion of D-glucosone to tri-O-isopropylidene D-glucosone hydrate these figures represent the formation of the osone in approximately 10% yield by the oxidation of D-fructose. Evans et al. (1928) reported a 24% yield of osone, estimated as D-glucose phenylosazone. This confirmation of the low yield of osone by this method makes it obvious that the procedure has little preparative value.

D-Glucose, L-sorbose, and D-xylose were oxidised with cupric acetate according to the method of Weidenhagen (1937). Since the products of oxidation were shown to contain unchanged starting material as well as osones they were condensed with acetone and from the products di-O-isopropylidene derivatives of of the un-oxidised sugars were removed by hot aqueous extraction. From the water-insoluble residues crystalline isopropylidene

derivatives of the corresponding osones, when D-glucose and L-sorbose were oxidised, were obtained by crystallisation from methanol in yields representing a 40% conversion of the sugars to the corresponding osones. The method of preparation may be carried out on a large scale and has provided a valuable source of the crystalline isopropylidene derivatives from which the pure osones may be obtained by hydrolysis.

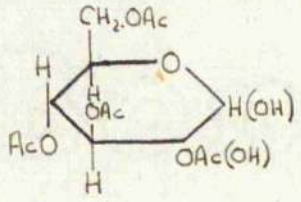
1.3.2. Action of Selenious Acid.

From D-fructose, by the method described by Dixon & Harrison (1932), D-glucosone was obtained in 8% yield; Dixon & Harrison (1932) did not quote a yield. These workers claimed that D-glucosone prepared in this manner regenerated the colour of Schiff's reagent and formed an addition compound with sodium bisulphite; the present author has been unable to confirm these observations with glucosone prepared either by this method or by any other.

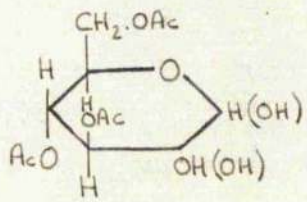
1.4. Indirect Syntheses of D-Glucosone.

1.4.1. Oxidation of 2:3:4:6-Tetra-O-Acetyl-2-Oxy-D-Glucal.

2:3:4:6-Tetra-O-acetyl-2-oxy-D-glucal was prepared from 2:3:4:6-tetra-O-acetyl-D-glucosyl bromide, in turn prepared directly from D-glucose by the method of Martos & Körösy (1950), by treatment with diethylamine according to the directions of Maurer (1929). Oxidation of the D-glucal derivative with perbenzoic acid (Stacey & Turton, 1946) gave crystalline 2:3:4:6-tetra-O-acetyl D-glucosone hydrate (VII) in comparatively poor yield, a large proportion of the starting material being recovered. The same D-glucosone derivative was also prepared by treatment of the non-crystalline products of the chlorination of tetra-O-acetyl-2-oxy-D-glucal, in ethereal solution, with silver carbonate and a little water, as described by Maurer (1929). Stacey & Turton (1946) reported the product of perbenzoic acid oxidation of the D-glucal derivative to differ from that of Maurer (1929) in that it did not exhibit mutarotation and also with regard to melting point. However, by either method of preparation, the samples of 2:3:4:6-tetra-O-acetyl D-glucosone hydrate obtained by the present author showed physical properties identical with those recorded by Maurer (1929) for this derivative. That the derivative contains an incipiently ionic hydrogen atom, as was claimed by Stacey & Turton (1946), was confirmed by titration with dilute alkali.



(VII)



(XII)

3:4:6-Tri-O-acetyl D-glucosone hydrate (XII) was prepared from the syrupy chlorination products of 2:3:4:6-tetra-O--acetyl-2-oxy-D-glucal by treatment with sodium bicarbonate and a small amount of water according to the method of Maurer

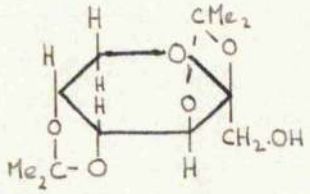
& Petsch (1931); the product showed physical properties identical with those given by these latter workers.

The present author has demonstrated unequivocally that 2:3:4:6-tetra-O-acetyl D-glucosone hydrate and 3:4:6-tri-O--acetyl D-glucosone hydrate are indeed derivatives of D-glucosone; such proof was not given by either Maurer and his coworkers or Stacey & Turton (1946) (see Part I, 1.4.1.). After short treatment with dilute alkali both derivatives reduced Fehling's solution at room temperature, readily formed D-glucose phenylosazone with phenylhydrazine in acetic acid solution, and gave a blue colour with Benedict's arsenophosphotungstic acid reagent in the presence of alkali-cyanide; catalytic deacetylation with metallic sodium in anhydrous methanol gave D-glucosone, identified chromatographically on paper employing the upper layer of a n-butanol-acetic acid--water (4:1:5) mixture as developer and triphenyltetrazolium chloride as identification reagent; treatment of either derivative with anhydrous acetone containing concentrated sulphuric acid gave a crystalline product identified as tri-O-isopropylidene D-glucosone hydrate, a derivative also obtained by similar treatment of D-glucosone (see Part II, 3.2.2.).

The preparation of D-glucosone by deacetylation of the crystalline acetates described above, although giving a product possessing a high degree of purity, involves the initial preparation of four intermediates (tetra-O-acetyl-D-glucosyl bromide, tetra-O-acetyl-2-oxy-D-glucal, the chlorination product of the latter, and the desired D-glucosone acetate), of which only the first may be obtained in good yield; in consequence, alternative methods of preparation, such as decomposition of the corresponding phenylosazone with benzaldehyde, are to be preferred.

1.4.2. Oxidation of 2:3-4:5-Di-O-*iso*Propylidene D-Fructose.

Oxidation of the free primary alcohol group of 2:3-4:5-di-O-*iso*propylidene D-fructose (XCVI) to an aldehyde group should provide an interesting di-O-*iso*propylidene derivative of D-glucosone in the non-hydrated form possessing a free aldehyde group and a 2:6-fructopyranose ring structure. However, attempts by the present author to carry out such an oxidation and isolate the product have met with no success.



(XCVI)

The use of selenious acid for the contemplated oxidation is precluded by the strong acidic conditions of such a method which would bring about hydrolysis of the *iso*propylidene groups of both the substrate and the product. It has been shown that no oxidation of di-O-*iso*propylidene D-fructose occurred on treatment with cupric acetate in methanol; this observation indicates the necessity of the presence of a free hydroxyl group (or, perhaps, a free carbonyl group) adjacent to the hydroxyl group to be oxidised by this reagent. Such a structural environment is also considered to be required for the oxidation of hydroxyl groups by Fenton's reagent (see Part I, 1.3.1.), and in consequence the use of this latter oxidant was not attempted.

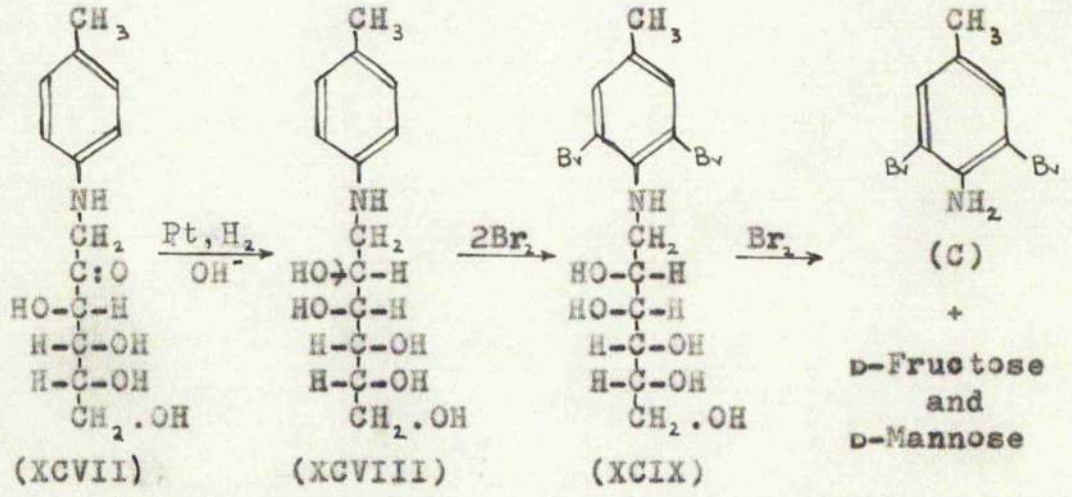
From the attempted oxidation of the fructose derivative, in chloroform solution, with manganese dioxide at room temperature for 6 days the starting material was recovered almost quantitatively; the small, non-crystalline residue did not regenerate the colour of Schiff's reagent, reduce Benedict's reagent, or form an addition compound with dimedone.

Oppenauer & Oberrauch (1949) described the use of *tert*-butyl chromate, a strongly acidic reagent, in non-polar

organic solvents for the selective and almost quantitative oxidation of primary alcohol groups to aldehyde groups. Attempts by the present author to oxidise di-O-isopropylidene D-fructose, in petroleum solution and using solid calcium carbonate as a buffer, with this reagent have met with little success; qualitative evidence of oxidation having occurred was obtained but isolation of the product was not achieved.

1.4.3. Oxidation of N-p-Tolyl-D-isoGlucosamine.

Weygand & Schaefer (1951) claimed that oxidation of N-p-tolyl-D-mannamine (N-p-tolyl-1-amino-1-desoxy D-mannitol) (XCVIII), prepared by catalytic hydrogenation of N-p-tolyl--D-isoglucosamine (XCVII) in alkaline or neutral solution (Weygand, 1940), with bromine in water gave D-mannose and D-fructose, identified chromatographically, and 2:6-dibromo--p-toluidine (C).



(XCVII) (XCVIII) (XCIX)

They showed 2:6-dibromo-N-p-tolyl-D-mannamine (XCIX) to be an intermediate, and postulated a mechanism for the formation of D-mannose. These workers found it difficult to explain the formation of fructose; it was suggested that (XCIX) might be further oxidised to 2:6-dibromo-N-p-tolyl-D-isoglucosamine, which on hydrolysis would yield D-fructose. However, Weygand & Schaefer (1951) were unable to identify any sugar products by treatment of N-p-tolyl-D-isoglucosamine (XCVII) with bromine in water. Following neutralisation and evaporation of the

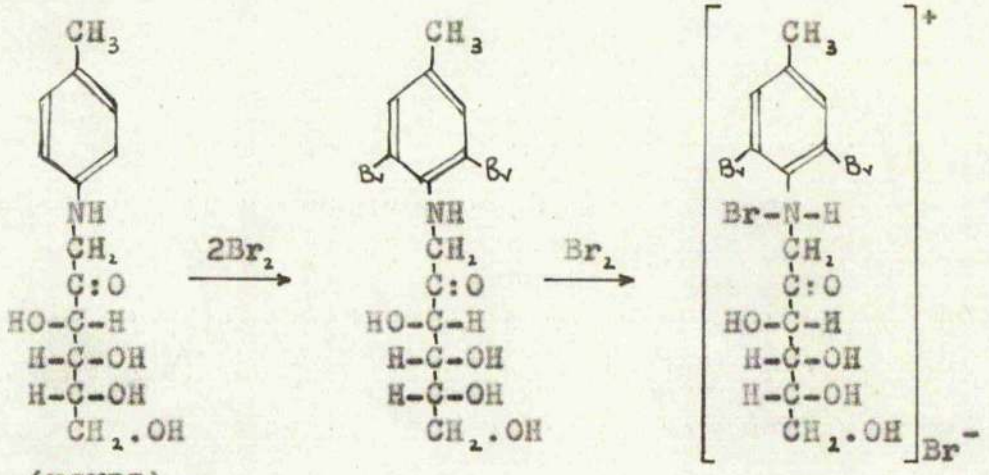
aqueous solution obtained by bromine oxidation of N-p-tolyl--D-mannamine these workers isolated the sugar products by extraction of the residue with hot pyridine; it is surprising that D-glucose, formed by epimerisation of the fructose and/or the mannose by the action of the base, was not identified on the chromatogram.

It was considered by the present author that oxidation of N-p-tolyl-D-isoglucosamine in the manner described above would yield D-glucosone; the inability of Weygand & Schaefer (1951) to identify such a product of this oxidation may be explained on the grounds that the glucosone was converted into kojic acid, or a derivative thereof, by the extraction procedure with pyridine.

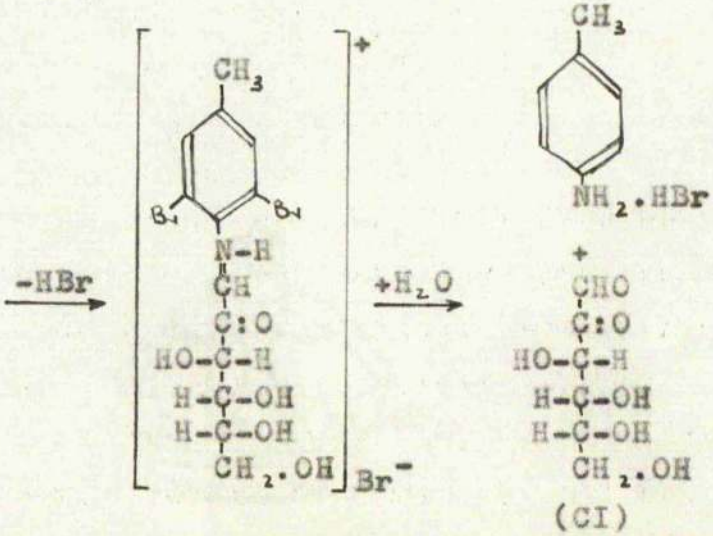
N-p-Tolyl-D-isoglucosamine was oxidised with bromine in water for 1 hour at room temperature. The almost colourless solution obtained after decantation from the brown tar of dibromo-p-toluidine, removal of excess bromine with a stream of nitrogen, and neutralisation with sodium hydroxide gave the following reactions: it reduced Fehling's solution at room temperature, gave a negative result with Seliwanoff's reagent (thus showing the absence of fructose), did not regenerate the colour of Schiff's reagent, and gave a blue colour with Benedict's arsenophosphotungstic acid reagent in the presence of alkali-cyanide; a small portion of the solution gave D-glucose phenylosazone on treatment with phenylhydrazine in acetic acid at room temperature; the presence of D-glucosone in the solution was confirmed by paper chromatographic analysis, employing the upper layer of a n-butanol-acetic acid-water (4:1:5) mixture as developing solvent and triphenyltetrazolium chloride as identification reagent. The neutral aqueous solution obtained as above was evaporated and the residue extracted with absolute ethanol; the syrup obtained by evaporation of the ethanolic extract was treated with anhydrous acetone containing concentrated sulphuric acid with the production, in

low yield, of crystalline tri-O-isopropylidene D-glucosone hydrate, identical with the product of similar treatment of D-glucosone (see Part II, 3.2.2.). It was thus demonstrated beyond doubt that D-glucosone is formed by this reaction. Attempts to adapt the method to the preparation of the crystalline isopropylidene derivative of D-glucosone on a large scale and in good yield were not successful, severe losses of the initial osone product being incurred during purification.

The following mechanism is proposed for the formation of D-glucosone (CI) by bromine oxidation of N-p-tolyl-D-isoglucosamine (XCVII), all compounds being represented in the open chain form:



(XCVII)



(CI)

A similar mechanism was postulated by Weygand & Schaefer (1951) for the formation of D-mannose by bromine oxidation of N-p-tolyl--D-mannamine.

1.5. The Purification of Osone Solutions.

The preparation of pure osones suitable for structural and metabolic investigations is a difficult task owing to their high reactivity and lack of crystallinity.

Initial purification of the solutions of osones obtained by decomposition of the corresponding phenylosazone with benzaldehyde or pyruvic acid was achieved by rigorous extraction with ether followed by treatment with charcoal thus removing unchanged carbonyl compound as well as last traces of unchanged starting material and by-product. The use of other organic solvents such as chloroform and ethylene dichloride for the extraction was shown to be of no advantage. Evaporation of the resulting solution under reduced pressure at 40° gave the osones as pale yellow syrups, which were extracted with hot ethanol to give solutions free of inorganic material. After further treatment with charcoal evaporation of the ethanolic extracts gave the unsubstituted hexosones in the form of white, hygroscopic brittle "froths".

Removal of inorganic contaminants from solutions of osones has also been achieved using ion-exchange resins; however, the use of strongly basic exchange resins, such as Amberlite I.R. 400-OH, for the removal of weak organic acids is precluded since it has been shown that strong retention of the osones on such resins occurs. Retention of the reducing sugars in general on the strongly basic resins has recently been reported by Roseman, Abeles & Dorfman (1952).

Several methods are described in the literature for the precipitation of osones when in admixture with other sugars and sugar derivatives. Thus Fischer (1889) precipitated osones as lead complexes in alkaline solution; such a method of purification is far from satisfactory for a variety of reasons. In the first place, the osone is exposed to alkaline conditions, that is, conditions under which these sugars are

very unstable , as shown by Petuely (1952) and the present author (see Part II, 2.2.3.); Morrell & Crofts(1902) showed that precipitation of osones in this manner from solutions containing other sugars gave products still contaminated with these sugars; the present author has shown severe decreases in the yield to be incurred by such a method of"purification". The criticisms of non-specificity and of serious loss of material may also be made of the precipitaion of osones from ethanolic solution by addition of ether (Morrell & Crofts, 1900). For the isolation and purification of osones prepared by direct oxidation of the corresponding aldoses ar ketoses chromatography on a cellulose column has been employed by the present author. Such a method of purification has also been applied to osones prepared by more specific methods (see Part II, 2.1.6.).

The last mentioned technique is not, however, applicable to large scale preparations. Purification of D- and L-glucosone and L-gulosone has been achieved by way of the corresponding crystalline tri-O-isopropylidene derivatives, which latter have been shown to be readily hydrolysed by dilute mineral acid with the production of chromatographically homogeneous solutions of the parent osones, (see Part II, 2.1.6.). By the treatment of the crude products of the oxidation of fructose and sorbose with cupric acetate, carried out on a 90g.-scale, with acetone containing concentrated sulphuric acid substantial yields of the tri-O-isopropylidene of the corresponding osones have been obtained. It is considered that only by way of such derivatives may osones suitable for structural and metabolic investigations be prepared.

1.6. Conclusions.

It is considered that as a general method of preparation of osones the decomposition of the corresponding phenylosazones with benzaldehyde or pyruvic acid is to be preferred. The availability and ease of preparation of the starting materials, the mild conditions of the reaction, the comparatively high yields, and the high degree of purity of the osone products are all reasons for such a preference. The hydrochloric acid method of decomposition, although providing osones in fair yield, uncontaminated with other sugars, has the great disadvantage of exposing the products to conditions in which partial dehydration of of the highly reactive osones, with the consequent production of furan derivatives as well as anhydrides analogous to those obtained from glucose and fructose under similar conditions, is a probability. At the same time such a method is not applicable to the preparation of partially methylated osones; short action of even dilute hydrochloric acid on, for example, tetra-O-methyl fructofuranose is known to bring about demethylation.

The preparation of osones by direct oxidation of the corresponding aldoses or ketoses results in poor yields of products severely contaminated with the products of further oxidation. However, by condensation with acetone followed by fractionation of the resulting isopropylidene derivatives such derivatives of D-glucosone and L-gulosone have been obtained in fair yield following oxidation of D-fructose and L-sorbose, respectively, according to the directions of Weidenhagen (1937). From the isopropylidene derivatives the pure osones may be obtained readily by hydrolysis with dilute mineral acid. Such a preparative procedure may be carried out on a very large scale.

Indirect methods of preparation have been shown to give very poor yields of osones, as well as involving the initial preparation of derivatives not always easily obtainable.

2. THE PROPERTIES AND REACTIONS OF GLUCOSONE.

2.1. Physical Properties.

2.1.1. Physical Form.

Neither D-glucosone nor any other osone, prepared by any of the methods described above, has been obtained crystalline in spite of numerous attempts employing a variety of solvents.

By the brisk evaporation of aqueous or alcoholic solutions under reduced pressure at 40° glucosone, as well as other unsubstituted osones, has been obtained as a white, hygroscopic, brittle "froth", which may be separated readily from the flask, broken up and further dried *in vacuo* at 20° over phosphorus pentoxide. The unsubstituted hexosones have also been prepared in the form of white, hygroscopic, amorphous powders by the addition of dry ether to their ethanolic solutions. An aqueous solution of such a preparation of D-glucosone was shown to be chromatographically identical with a similar solution of the "froth".

On cooling a solution of D-glucosone in hot glacial acetic acid the osone was precipitated as a cream, hygroscopic, amorphous solid; chromatographic analysis of an aqueous solution of this solid indicated the presence of a high proportion of short chain decomposition products.

2.1.2. Molecular Formula.

Elementary analysis for carbon and hydrogen of D-glucosone prepared as a "froth" and dried to constant weight *in vacuo* at 20° gave results consistent with a molecular formula of $C_6H_{12}O_7$; in view of the properties and reactions of the osone such results are interpreted as indicating the existence of the sample as a monohydrate, $C_6H_{10}O_6.H_2O$. This view is supported by quantitative estimation of solutions of the "froth" employing an acid hydrolysate of crystalline tri-*O*-isopropylidene

D-glucosone hydrate as a standard. (see Part II, 2.2.5.)

2.1.3. Solubility Properties.

D-Glucosone is very readily soluble in water and methanol, less soluble in ethanol; the osone is very sparingly soluble in cold glacial acetic acid, dioxan, and acetone, and insoluble in chloroform, ether, light petroleum, and benzene.

2.1.4. Optical Properties.

Solutions of D-glucosone have been shown to exhibit mutarotation. Thus, a 9.27% aqueous solution of the "froth", shown, analytically, to be a hydrate of D-glucosone, showed an initial specific rotation of -10.58° which increased to a constant value of +4.21° after 150 hours (see Fig. 1.). The exhibition of mutarotation indicates the presence of at least one lactol ring in the molecule and from the direction of change in rotation it is suggested that the greater proportion of the "froth" is the β-anomer of such a ring structure.

The mutarotations of many sugars follow the first-order equation (Hudson, 1903; Isbell & Pigman, 1937) and this conformation makes it probable that the main constituents of the equilibrium solutions of these sugars are the α- and β--pyranose modifications. For such sugars a plot of log(rotation at time t - final equilibrium rotation) against time results in a straight line. A number of important sugars, including D-galactose, D-talose, and L-ribose, exhibit mutarotations which do not follow the first-order equation, and a "first-order plot" for such sugars results in a curve; the deviation of the curve from a straight line is an indication of the lack of conformity of the mutarotation with the first-order equation. A "first-order plot" for D-glucosone (Fig. 2.) results in a curve very similar to that obtained by Isbell & Pigman (1937) for the mutarotation of α-D-talose, and demonstrates the complexity of the mutarotation of the osone.

Fig. 1. Mutarotation of D-Glucosone at 18° (c, 9.27 in water).

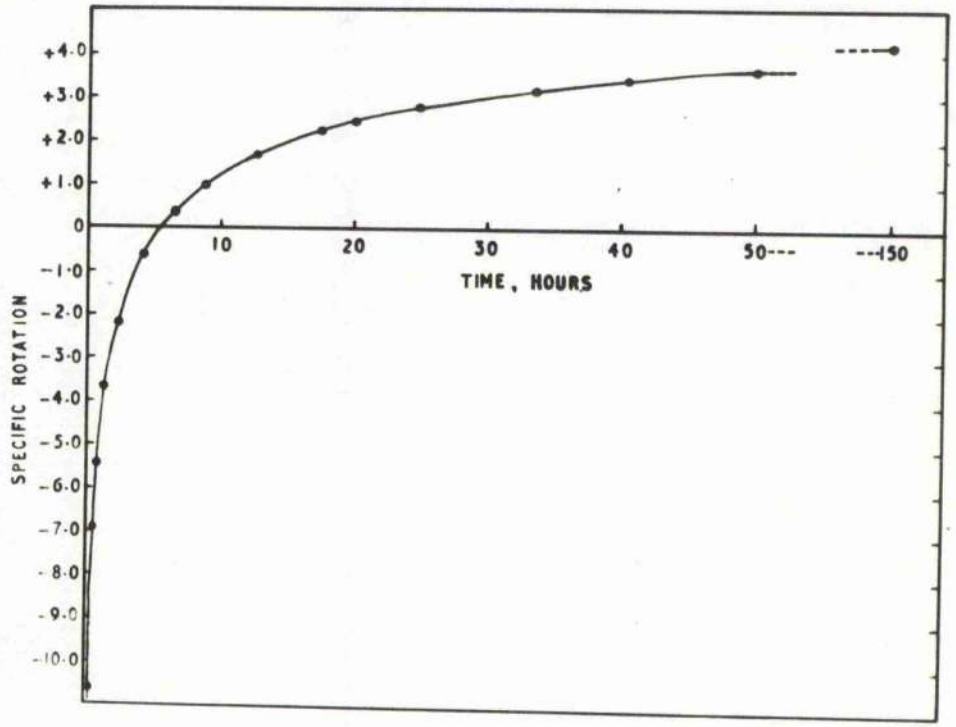
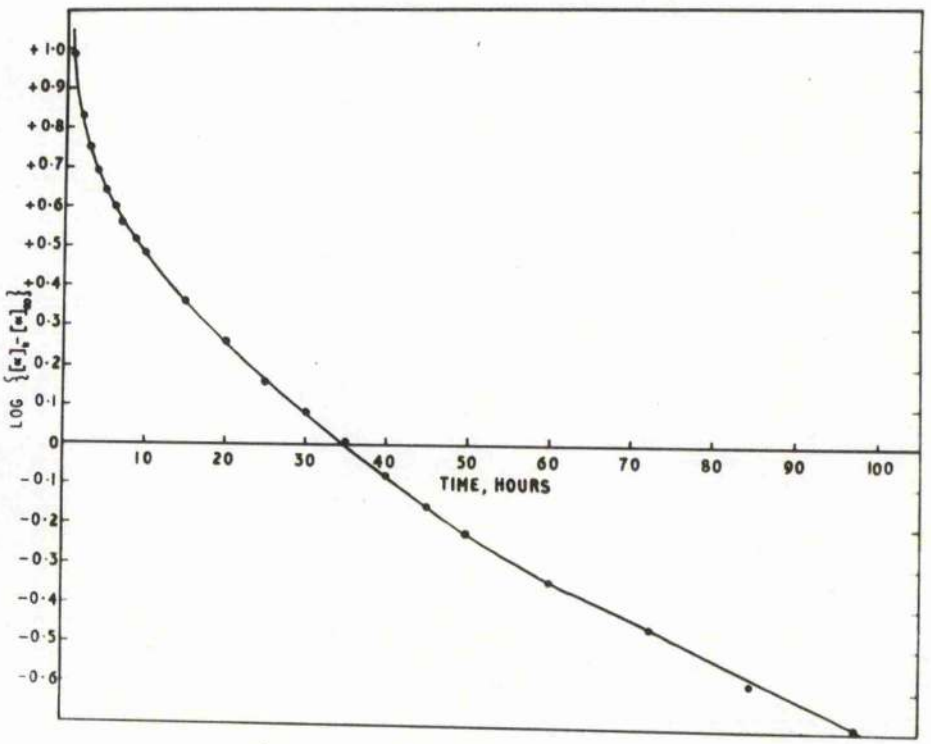


Fig. 2. First-order Plot of Mutarotation of D-Glucosone at 18° (c, 9.27 in water).



In general, the mutarotations which cannot be expressed by the first-order equation conform to equations derived on the assumption of three or more components in the equilibrium mixture (Riiber & Minsaas, 1926; Smith & Lowry, 1928; Isbell & Pigman, 1937). The mutarotation reactions which follow such equations may be considered to consist of two simultaneous or consecutive reactions, one of which is slow and represents the α,β conversions between pyranose isomers, and the other of which is rapid and possibly represents pyranose-furanose interconversions (Isbell & Pigman, 1937, 1938). Thus, the complexity of the mutarotation of D-glucosone, which shows both a fast and a slow reaction (see Fig. 2.), may perhaps be explained in terms of these types of interconversion. However, consideration must be given to other factors probably contributing to the production of a complex mutarotation.

During the course of mutarotation of the osone solution no change in reducing power or in the content of osone, estimated as D-glucose 2:4-dinitrophenylosazone (see Part II, 2.2.5.) could be detected, and no change in pH was observed. However, paper chromatographic analysis (see Part II, 2.1.6.) indicated the occurrence of certain structural interconversions. It was shown that partial concentration of a homogeneous solution of the osone, prepared by hydrolysis of tri-O-isopropylidene D-glucosone hydrate, led to the formation of a second component considered to be a stable polymer of the osone; a chromatogram of a fresh solution of the "froth", obtained by complete concentration, showed the presence not only of the pure osone and the stable polymer but also of a third component of intermediate R_F value. Chromatographic analysis during the course of mutarotation showed the gradual disappearance of this central spot and it is proposed that this component is an unstable polymer which depolymerises in aqueous solution. The initial presence of this unstable polymer as well as the pure osone and the stable polymer in the system would account for the exhibition of complex mutarotational

behaviour by a solution of the "froth". Also, as a result of a study of the reactions and ultraviolet absorption spectrum of solutions of the osone (see Part II, 2.1.5.) it is proposed that D-glucosone hydrate is not stable in solution, a structure containing a free carbonyl group at C_2 being formed in small proportion (see Part II, 4.); such a conversion would further contribute to the production of a complex mutarotation.

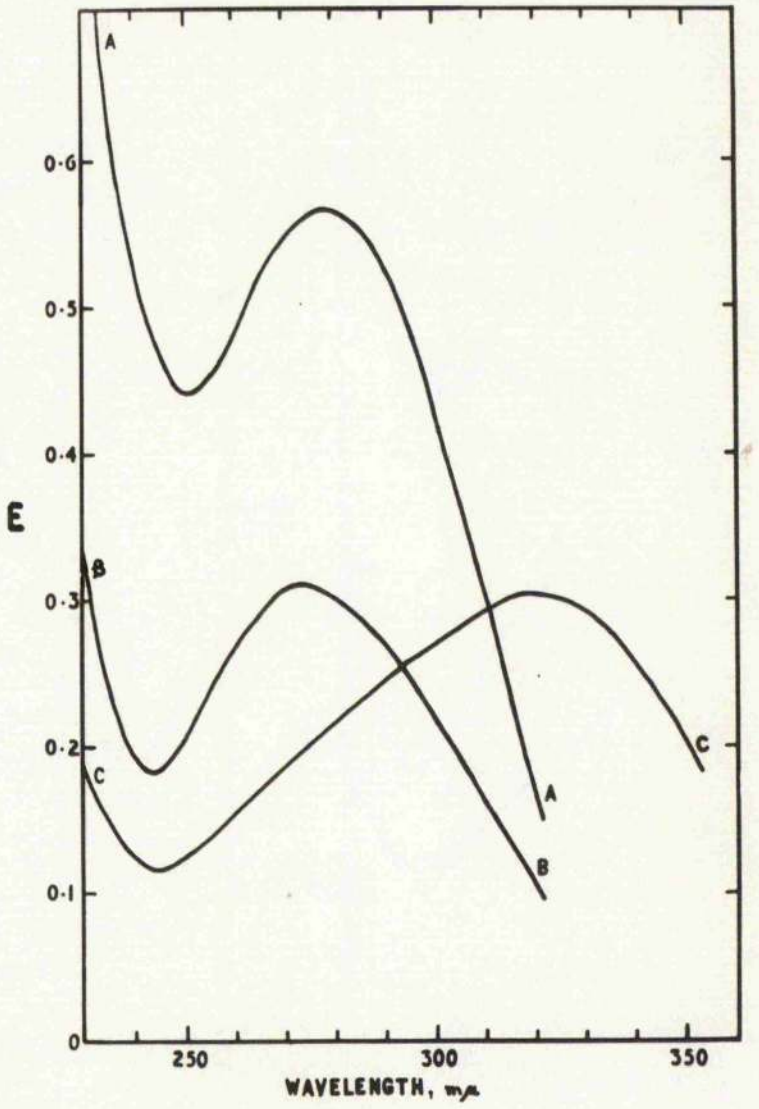
Thus, the complex mutarotation of D-glucosone is not to be ascribed to a simple interconversion of the pyranose-furanose type but rather to the ready polymerisation of the highly reactive molecule, and the instability in solution of the hydrated form of the monomer.

2.1.5. Spectrophotometric Analysis.

In view of the results of the work of Bednarczyk & Marchlewski (1938) on the absorption of ultraviolet light by solutions of crude D-glucosone (see Part I, 2.1.5.) similar investigations have been carried out by the present author on the pure osone in aqueous solution at different pH levels.

Chromatographically homogeneous solutions of D-glucosone, prepared by hydrolysis of tri-O-isopropylidene D-glucosone hydrate with 0.1N-sulphuric acid, were examined with the Unicam ultraviolet spectrophotometer, model S.500; the results are presented graphically - see Fig. 3. Curve A represents the absorption spectrum of a 0.3% solution of the osone in 0.04N sulphuric acid, i.e. a solution of pH 1.5; an absorption maximum was observed at a wavelength of 278mμ. and a minimum at 250mμ. Neutralisation of this solution with ion-exchange resin gave a solution of pH 7.0 whose absorption spectrum is represented by curve B; it may be seen that both absorption maximum and minimum were displaced towards the shorter wavelengths, being at 277 and 244mμ. respectively. Such a displacement could be accounted for by the necessary alteration in slit width used with the instrument as well as the decrease in concentration of

Fig. 3. Ultraviolet Absorption Spectra of Solutions of D-Glucosone.



A: Aqueous solution of D-glucosone, pH 1.5

B: Aqueous solution of D-glucosone, pH 7.0

C: Aqueous solution of D-glucosone, pH 12.5

the solution due to retention of the osone on the ion-exchange resin, apart from possible effects of the increase in pH value. The neutral solution was made alkaline (pH 12.5) by the addition of sodium hydroxide solution and after standing at room temperature for 10 minutes examined in the spectrophotometer. The absorption spectrum of this alkaline solution (curve C), showing an absorption maximum at 318mμ. and a minimum at 245mμ., is almost identical with that described by Stacey & Turton (1946) for kojic acid in alkaline solution and for the product of alkaline hydrolysis of their specimen of 2:3:4:6-tetra-O-acetyl D-glucosone hydrate - see Table 1.

Compound.	Max.(mμ)	Min.(mμ)	Reference.
L-Sorbose	278	243	B. & M. (1938)
D-Glucosone	297.5	251	B. & M. (1938)
D-Glucosone (pH 1.5)	278	250	J.A.F.
D-Glucosone (pH 7.0)	277	244	J.A.F.
D-Glucosone (pH 12.5)	318	245	J.A.F.
Kojic Acid (alk. soln.)	315	265	S. & T. (1946)
Alkaline hydrolysate of tetra-O-acetyl D-glucosone hydrate	315	249	S. & T. (1946)

A number of conclusions may be drawn from these results. Firstly, the absence of an absorption maximum at 285mμ in the spectrum of the acid solution of the osone, which was prepared by dilution of the acid hydrolysate of tri-O-isopropylidene D-glucosone hydrate, confirms the absence of 5-hydroxymethyl-furfuraldehyde, previously indicated by chromatographic analysis, in such hydrolysates.

Secondly, the presence of an absorption band in the region 277-278mμ. for both neutral and acid solutions of the osone suggests the presence of a free carbonyl group in the molecule. Thus the results obtained by the author for D-glucosone in neutral solution approximate much more closely to those reported

by Bednarczyk & Marchlewski (1938) for pure L-sorbose, a sugar known to exist in solution partially in the free keto form, than do the results of these latter workers for their crude specimen of the osone. Bednarczyk & Marchlewski (1937) and, more recently, Eustigneev & Nikiforova (1950) have shown neutral solutions of the aldohexoses to exhibit no selective absorption in the ultraviolet region and it may therefore be concluded that the absorption band corresponding to a free carbonyl group exhibited by D-glucosone is due to the presence of such a group on C_2 and not C_1 of the osone molecule. Such a conclusion is further supported by the observations of Pacsu & Hiller (1948) who reported that solutions of glucose and arabinose only in 50% sulphuric acid showed selective absorption corresponding to a free carbonyl group; such absorption was considered to be due to the presence of the open chain forms of the sugars in such solution since the absorption band disappeared on neutralisation. The characteristic carbonyl absorption band was observed in neutral as well as acid solutions of the osone by the present author; it is therefore proposed that D-glucosone, which in the solid state exists as a hydrate (see Part II, 2.1.1.), is partially present in a form containing a free carbonyl group at C_2 when in aqueous solution, thus explaining the ready polymerisation of the osone. It is interesting to recall that Niederhoff (1929) reported that a solution of 2-oxo-D-gluconic acid showed no selective absorption of ultraviolet light corresponding to a free carbonyl group and concluded that the carbonyl group at C_2 in the acid was involved in a lactol ring; however, hydration of this group, stabilised by the presence of the adjacent carboxyl group, is an alternative interpretation of such results and is supported by the fact that the free acid does not exhibit mutarotation (see Part II, 4.).

Thirdly, the observation of an absorption spectrum closely resembling that of kojic acid for dilute alkaline

solutions of the osone indicates quite clearly that such treatment with alkali causes widespread changes in the molecular structure of the osone. Such an observation also agrees with the results of experiments on the treatment of D-glucosone with a variety of alkaline reagents to give solutions giving qualitative tests for kojic acid (see Part II, 2.2.3.).

Fourthly, since no absorption bands corresponding to the enediol system characteristic of the reductones and ascorbic acid and its analogues were observed for acid and neutral solutions of the osone a number of conclusions drawn by other workers in the field of osone chemistry may be questioned. Enediolic structures are characterised by an absorption maximum in the region 240-265mμ. for acid solutions which is displaced to 265-290mμ. for solutions of pH 6-12; in stronger alkaline solution an absorption band in the region 298-320mμ. may be observed (see Table 2.). Carpéni (1938b) has interpreted such results as being due to the presence of the undissociated molecule, the univalent ion, and the divalent ion, respectively, in solution in the three pH ranges described above. In contrast, the present author has shown that both acid (pH 1.5) and neutral solutions of D-glucosone exhibit only an absorption maximum at 277-278mμ., corresponding to a free carbonyl group; such results provide experimental confirmatory evidence against the hypothesis of Brüll (1937) that the osones may be considered as reduction products of ascorbic acid and its analogues, a proposal which may also be refuted on theoretical grounds (see Part I, 4.). Petuely (1952) claimed to demonstrate a 12% enolisation of D-glucosone by treatment with 0.1N-mineral acid; the absorption spectrum observed by the present author does not confirm such enolisation, acid and neutral solutions of the osone exhibiting approximately the same absorption maximum. Petuely (1952) also claimed to show 30% enolisation of glucosone by 0.1N-sodium hydroxide solution of which 10.8% was accounted as redutones (see Part I, 2.2.4.). The observation by the present author

Table 2.

Compound	Max.(mμ.)	Reference
L-Ascorbic Acid(acid or conc. soln.)	245.0	Herbert et al. (1933)
L-Ascorbic Acid(alk. soln.)	265.0	
L-Ascorbic Acid(aq. soln.)	252.0	Mohler & Lohr (1938)
L-Ascorbic Acid(weak alk. soln.)	264.0	
L-Ascorbic Acid(pH 0.0-3.0)	242.5	
(pH 5.5-11.5)	264.5	Carpéni (1938b)
(pH 12.5-14.0)	298.0	
D-Araboascorbic Acid(pH 0.0-3.0)	242.0	
(pH 5.5-11.5)	264.5	Carpéni (1938b)
(pH 12.5-14.0)	299.0	
Reductone(pH 0.0-4.0)	267.5	
(pH 6.0-12.0)	291.0	Carpéni (1938b)
(pH 13.4-14.0)	320.0	
Reductic Acid(pH 0.0-3.5)	265.0	
(pH 6.0-12.0)	279.0	Carpéni (1938b)
(pH 13.5-14.0)	314.0	
Reductic Acid(aq. soln.)	267.0	Mohler & Lohr (1938)
Reductic Acid(weak alk. soln.)	279.0	

of an absorption maximum at 318mμ. for D-glucosone in alkaline solution (pH 12.5) does not contradict such a proposal since Carpéni (1938b) has reported a maximum at 320mμ. for a solution of reductone of comparable alkalinity. At the same time, however, Stacey & Turton (1946) have recorded an absorption maximum at 315mμ. for alkaline solutions of kojic acid, a well established transformation product of acetates of glucosone by treatment with alkaline reagents (see Part I, 2.2.4.); Petuely (1952) gave no consideration to the possibility of the formation of kojic acid by treatment of the free osone with alkali. In addition, alkaline solutions of glucosone, on adding a few drops of ferric chloride solution, give a cherry red colour characteristic of a γ-pyrone structure and not the deep blue colour given by reductones (see Part II, 2.2.4.). Thus, from the ultraviolet absorption data it is impossible to differentiate between reductone and kojic acid formation but the weight of evidence, obtained by alterntive methods of investigation, is in favour of the latter being the main product of the action of alkali on glucosone, possibly formed by way of a rapid enolisation.

2.1.6. Chromatographic Analysis.

The behaviour of D-glucosone on the paper chromatogram has been fully investigated in order to establish the degree of homogeneity of different preparations and to provide a means of detection and identification of the osone when in admixture with other sugars or in biological material.

A large number of developing solvents and identification methods have been employed, using both the descending and ascending techniques on a variety of Whatman papers. The most successful methods for rendering the osone, as well as other sugars, visible on the developed chromatograms were:
a) a modification of the technique of Trevelyan, Procter & Harris (1950), using triphenyltetrazolium chloride; on warming

at 60° the sugars appeared as red spots on a white ground in the following order: glucosone, fructose, glucose, thus providing a means of identification of the osone other than by measuring R_F value.

b) aniline hydrogen phthalate in glacial acetic acid; on heating at 100° the following order of appearance was noted: glucosone, glucose, fructose.

c) a modification of the silver nitrate technique due to Trevelyan, Procter & Harris (1950).

An identification technique by which glucosone is made visible, as well as 2-oxo-aldonic acids and glyoxal derivatives, but not the ordinary reducing sugars, was evolved by an adaptation of the reaction with Benedict's arsenophosphotungstic acid reagent in the presence of alkali-cyanide. The dried, developed chromatogram was passed through a solution of the reagent in aqueous acetone, dried, and passed through a solution of sodium cyanide in 0.05N-ethanolic sodium hydroxide; the osone appeared immediately as a blue spot on a white ground. Only on drying and heating was fructose made visible, and even under these conditions glucose did not appear. The use of this identification reagent together with measurement of R_F value provides a diagnostic procedure for osones when in admixture with other sugars.

For the determination of accurate R_F values D-glucosone was prepared by hydrolysis of tri-O-isopropylidene D-glucosone hydrate with 0.1N-sulphuric acid; the hydrolysate was neutralised with cation-exchange resin to give an approximately 0.4% neutral aqueous solution of the osone. Such a solution invariably gave a single spot on the chromatogram employing a large number of different developing systems, but, in most, "tailing" of the osone spot was observed. R_F values for the osone, measured from chromatograms on Whatman No. 1 paper developed at 18°, are recorded in Table 3., and are compared with those observed for some other sugars of biological importance; an

Table 3.

Developer	R_f Values			
	Glucosone	Glucose	Fructose	Lactose
Upper phase of n-butanol--acetic acid-water (4:1:5)	0.17 (T)	0.18 (C)	0.23 (C)	0.06 (C)
Upper phase of n-butanol--acetic acid-water (4:5:1)	streak	0.24 (E)	0.29 (E)	0.09 (E)
Phenol-water (4:1)	0.25 (E)	0.36 (C)	0.50 (C)	0.30 (C)
n-Butanol, satd. with water	0.05 (E)	0.07 (C)	-	-
n-Butanol, satd. with water and solid oxalic acid	0.07 (C)	0.08 (C)	-	-
n-Butanol-ethanol-water (40:11:19)	0.16 (T)	0.13 (C)	-	-
Methanol-ethanol-water (45:45:10)	0.51 (T)	0.43 (T)	-	-
Dioxan-methanol (85:15)	streak	0.21 (T)	-	-
70% Aqueous ammonium sulphate	0.94 (C)	0.97 (C)	-	-

[(C) = round, compact spot
(E) = elliptical spot
(T) = elliptical spot with "tailing"]

indication of the shape of the osone spot obtained with each solvent system is made.

Hapson & Partridge (1949) eradicated the "tailing" of ascorbic acid on the paper chromatogram by the use of developers containing volatile organic acids, such as the upper layer of a n-butanol-acetic acid-water (4:1:5) mixture; using this developer Partridge (1948) reported dehydroascorbic acid to give an elliptical spot. The present author has shown D-glucosone to exhibit severe "tailing" with this developer - see Plate 1., a negative on contact paper of a chromatogram on Whatman No.1 paper of various samples of D-glucosone compared with D-glucose, D-fructose, and lactose. Macek & Tadra (1952) considered that even higher concentrations of organic acid were required in the developer to prevent the "tailing" of 2-oxo-aldonic acids, and recommended the use of the upper layer of a n-butanol-acetic acid-water (4:5:1) mixture; they postulated that prevention of "tailing" in such a system was due to suppresion of the ionisation of the 2-oxo acids by the high percentage of acetic acid in the developer. It was considered by the present author that since the possibility exists that glucosone, in solution, is present in a hydrated form containing an incipiently ionic hydrogen atom, the "tailing" of the osone in most developers might be due to ionisation. However, using the system of Macek & Tadra (1952), the "tailing", far from being alleviated, was increased, with the production of a long streak; thus, ionisation as an explanation of the "tailing" is precluded.

The inclusion of benzene in chromatographic developers is recognised as assisting in the production of compact spots; such an effect, however, was not observed with regard to glucosone when benzene was added to the extent of 10% to a n-butanol-acetic acid-water (4:1:5) mixture, the only result being a decrease in R_F value.

The only solvent systems in which glucosone was obtained

Plate 1.

Chromatogram of solutions of:

1. Glucose
2. Fructose
3. Lactose
4. Glucosone (unpurified "froth")
5. Glucosone (concentrate of column eluate)
6. Glucosone (hydrolysate of tri-O-isopropylidene derivative)

Paper: Whatman No. 1.

Developer: Upper phase of n-butanol-acetic acid-water (4:1:5) mixture.

Identification reagent: Triphenyltetrazolium chloride.

Temperature: 18°.

Time: 16 hours.

(4)

as a round compact spot were n-butanol saturated with water and solid oxalic acid, and 70% aqueous ammonium sulphate solution. The former developer was used by Isherwood (1953) for the chromatography of ascorbic acid and related compounds; he reported that in most other solvents severe "tailing" occurred due to oxidation of the ascorbic acid during development, and that such oxidation was inhibited by the presence of oxalic acid. Watanabe (1937) had previously observed the stability of ascorbic acidin aqueous oxalic acid solution. It should be noted that Mapson & Partridge (1949) considered the "tailing" of ascorbic acid to be due to ionisation as well as the instability of the enediol, and was to be prevented by the use of acid developers. If indeed the"tailing" of ascorbic acid is attributable to oxidation, prevented by the presence of oxalic acid, then the fact that compact spots were obtained for the osone using the developer of Isherwood (1953)suggests that the "tailing" of this sugar in most other systems is also due to partial oxidation during development; the characteristic reactivity of the osone makes such an explanation feasible. An alternative explanation of the "tailing" of glucosone is the instability of the hydrate in solution, as shown by chemical reactions and ultraviolet absorption spectrophotometry (Part II, 2.1.5.).

Although compact spots were obtained using the developers described immediately above good separation of the osone from other sugars was not achieved, even on multiple development. For the purpose of resolution of mixtures of sugars containing the osone the most effective developers examined were those containing phenol (see Table 3.); the osone appeared as an ellipse, which on long development exhibited some "tailing". Plate 2. shows the separation of D-glucosone, D-glucose, D-fructose and lactose on Whatman No.1 paper at 18° using phenol-water (4:1) as developer and aniline hydrogen phthalate in glacial acetic acid as spray reagent. Apart from lactose, which has an R_F value in this system close to that of the osone,

Plate 2.

Chromatogram of solutions of:

1. Glucose
2. Fructose
3. Lactose
4. Glucosone (unpurified "froth")
5. Glucosone (concentrate of column eluate)
6. Glucosone (hydrolysate of tri-O-isopropylidene derivative)
7. Mixture of 1., 2., 3., and 6.

Paper: Whatman No. 1.

Developer: Phenol-water (4:1).

Identification reagent: Aniline hydrogen phthalate in glacial acetic acid.

Temperature: 18°.

Time: 36 hours.

resolution of the sugars may be considered to be adequate.

Employing neutral developers such as n-butanol-ethanol--water (40:11:19) and methanol-ethanol-water(45:45:10) severe "tailing" or the production of a long streak was observed for glucosone, from which it may be concluded that the "tailing" in acidic developers is not caused by partial decomposition of the osone by the action of the acid. Using an anhydrous developer, dioxan-methanol (85:15), a streak was again produced.

The use of basic developers containing pyridine, collidine, ammonia etc., which have been employed with success for the chromatography of other sugars, is precluded since it was shown that partial decomposition of glucosone occurs in these solvents with the formation of a product giving qualitative tests for kojic acid.

The results of these investigations may be summarised as follows: D-glucosone, prepared by hydrolysis of its tri-O--isopropylidene derivative, behaves as a single entity on the paper chromatogram; glucosone, in the presence of other sugars, may be identified and detected chromatographically by measurement of R_F value, by observation of the rate of appearance of the osone spot compared with those of the other sugars using triphenyltetrazolium chloride or aniline hydrogen phthalate as identification reagents, and by the use of the specific arsenophosphotungstic acid reaction; the "tailing" of the osone with the majority of developers may be ascribed not to decomposition by acid or to ionisation but to partial oxidation.

D-Glucosone, behaving as a single entity on the paper chromatogram, was also obtained by the elution of the osone, prepared by the benzaldehyde method and applied in concentrated methanolic solution, from a cellulose column with acetone-water (9:1), after the method developed by Hough, Jones & Wadman (1949) for other sugars. The osone was eluted as a broad band and identified by reaction with the arsenophosphotungstic acid

reagent in the presence of alkali-cyanide. Concentration of either this homogeneous eluate or the solution of D-glucosone obtained by hydrolysis of the crystalline isopropylidene derivative to thin syrups which were then dissolved in water to give approximately 1% solutions provided samples of the osone which were shown, chromatographically, to be heterogeneous. Thus, using a variety of developers, two spots were obtained with these diluted concentrates; one spot proved to be identical with single"tailing" spot of the homogeneous samples described previously, while the other (designated "polymer" in Table 4.) was round and compact and of lower R_F value, approximately the same as that of the disaccharide lactose - see Table 4. With the three identification techniques discussed previously the spot of lower R_F value was rendered visible more slowly than was the true osone spot but more rapidly than was fructose.

Table 4.

Developer	R_F Values		
	Glucosone	"Polymer"	Lactose
Upper layer of n-butanol--acetic acid-water (4:1:5)	0.17(T)	0.07(C)	0.06(C)
n-Butanol-ethanol-water (40:11:19)	0.16(T)	0.05(C)	0.06(C)
Methanol-ethanol-water (45:45:10)	0.51(T)	0.29(C)	-

The heterogeneous chromatogram was obtained even when the pure osone solutions were concentrated under reduced pressure in an atmosphere of nitrogen, thus suggesting that the formation of the second component of lower R_F value is to be associated only with concentration and not oxidation. From its reaction with indicator sprays such as bromothymol blue this second component was shown not to be acidic in nature, and its

R_F value does not correspond with that of the expected product of the oxidation of D-glucosone, namely, 2-oxo-D-gluconic acid; compounds liable to be formed by rearrangement of the osone molecule, such as kojic acid and D-gluconic acid have been shown not to be made visible on the chromatogram using triphenyltetrazolium chloride; from its R_F value the second component is shown not to be identical with such products of glucosone degradation as a pentose, or lower sugar, furfural, or laevulinic acid.

By further elution of the cellulose column, or by elution of large scale paper chromatograms of the concentrates described above, solutions of the second component were obtained; concentration of such solutions yielded a syrup which could not be crystallised. A solution of the syrup gave a positive reaction with Molisch's reagent, reduced Fehling's solution only on warming, and gave a blue colour with the arsenophosphotungstic acid reagent in the presence of alkali-cyanide only on standing; this solution was also shown to be chromatographically homogeneous. Thus, like the sample of glucosone prepared by hydrolysis of the isopropylidene derivative, that providing the second spot is a single structural entity and not a component of a dynamic equilibrium. From its mode of formation, that is, by concentration of solutions of the pure osone, its lack of identity with the possible products of glucosone oxidation, rearrangement, or degradation, its chemical properties, and its R_F value it is speculated that the compound is a dimer of D-glucosone, possibly analogous to the well-known dianhydrides readily obtained from fructose; hereafter the compound is referred to as "polymer".

The R_F value of the "polymer" for chromatograms developed with the upper layer of a n-butanol-acetic acid-water (4:1:5) mixture is in close agreement with the figure recorded by Petuely(1952) for the spot on similar chromatograms of unpurified D-glucosone which was considered by him to be the mono-lactol ring component of a dynamic equilibrium; the R_F value of pure

D-glucosone observed by the present author is identical with that given by Petuely (1952) for what he considered to be a di-lactol ring component of the equilibrium. That two spots are obtained on chromatograms of solutions of unpurified D-glucosone, prepared by the benzaldehyde method, in the manner described by Petuely (1952), has been confirmed by the present author; such chromatograms are identical with those obtained with solutions of the concentrates of the pure osone - see Plate 1., page 116.

Since it has been shown that solutions of previously purified D-glucosone are chromatographically homogeneous, and that the "polymer" produced on concentration of such solutions may be isolated and similarly shown to be homogeneous, it is obvious that the osone and the "polymer" are not two components of a dynamic equilibrium; they are, in fact, two distinct structures, the "polymer" being formed from the osone either during concentration of pure solutions of the latter or, in the case of unpurified samples of the osone, during the initial preparative procedure. The hypothesis of Petuely (1952) that glucosone exists in solution as two types of isomer in dynamic equilibrium is therefore untenable; his conclusions were drawn from a chromatographic study of impure glucosone. The present author's own deductions are supported by the observation that unpurified 3:5:6-tri-O-methyl D-glucosone, a compound incapable of containing two furanose or pyranose rings and yet, structurally, capable of polymerising, also gives a pair of spots on the paper chromatogram. It might be proposed that the two spots obtainable from glucosone do represent two different ring forms of the sugar, one containing a single furanose or pyranose ring and a hydrated keto group at C_2, and the other that same ring together with a 1:2-anhydro ring analogous to that considered Maurer & Petsch (1933) to be present in tetra-O-benzoyl D-glucosone (see Part I, 1.4.1.); such a structural modification could also theoretically exist for the tri-O-methyl

osone described above. However, since in the case of D-glucosone it has been shown that both the pure osone and the "polymer" are stable to hot mineral acid such an argument cannot hold.

Chromatograms of fresh aqueous solutions of the "froth" obtained by rigorous concentration of solutions of pure D-glucosone showed the presence of three components. Thus, apart from the"tailing" spot of the pure osone and the round compact spot of the"polymer" a third spot of intermediate R_F value was rendered visible - see Plate 3. On standing the third component gradually disappeared from these solutions and was not detectable on the chromatogram; it is proposed that the third spot is caused by the presence of an unstable polymer of the osone which depolymerises in aqueous solution, thus accounting, in part, for the complex mutarotations exhibited by aqueous solutions of the "froth" (see Part II, 2.1.4.). Plates 3.-6. (see page 124.) show chromatograms of an aqueous solution of D-glucosone "froth" made during the course of the mutarotation of the solution; these chromatograms demonstrate the gradual disappearance of the unstable polymer with the production of a solution chromatographically identical with the solution obtained by partial concentration of the pure osone.

Plate 3.

Chromatogram of solutions of:

1. Glucose
2. Fructose
3. Lactose
4. Glucosone (unpurified "froth", immediately after solution)
5. Glucosone (concentrate of column eluate)
6. Glucosone (hydrolysate of tri-O-isopropylidene derivative)

Paper: Whatman No. 1.

Developer: Upper phase of n-butanol-acetic acid-water (4:1:5) mixture.

Identification reagent: Triphenyltetrazolium chloride.

Temperature: 18°.

Time: 16 hours.

Plate 4.

As Plate 3. except:

4. Glucosone (unpurified "froth", 15 hours after solution)

Plate 5.

As Plate 3. except:

4. Glucosone (unpurified "froth", 60 hours after solution)

Plate 6.

As Plate 3. except:

4. Glucosone (unpurified "froth", 250 hours after solution)

2.1.7. Ionophoretic Analysis.

Attention has recently been focussed on the use of filter-paper ionophoresis as a technique complementary to that of chromatography for structural studies and analysis of carbohydrates (Jaenicke, 1952; Consden & Stanier, 1952; Foster, 1952, 1953; Woodin, 1952; Foster & Stacey, 1953). Ionophoretic migration of certain neutral sugar derivatives occurs at an alkaline pH in the presence of borate ions and results from the formation of weak negatively charged complexes. Consden & Stanier (1952) have shown that the relative distance travelled by a sugar under standard conditions of applied voltage varies with the stereochemical configuration of the sugar and the pH value of the borate buffer employed. By the choice of appropriate conditions (pH and time) these workers resolved various sugar mixtures, and showed that borate-complex formation was undoubtedly taking place and giving rise to mobility since in buffers not containing borate there was no migration; clear separations were obtained only in borate buffers of pH greater than 8.0. Investigations of an extension of the method to the separation of disaccharides as well as of the mode of interaction of borate ions with carbohydrates was carried out by Foster (1953).

A study of the ionophoretic behaviour of D-glucosone, as compared with that of D-glucose and of D-fructose, in borate buffers of varying pH has been made by the present author. Pure D-glucosone was obtained by acid hydrolysis of its crystalline <u>iso</u>propylidene derivative and was applied in 1% aqueous solution, with the aid of a small brush, to 2 cm.-wide strips of filter paper (Whatman 3MM), previously dipped in buffer and blotted, as were similar solutions of the other sugars; as a control a neutral substance, creatinine, similarlarly applied to a filter-paper strip, was included in each "run". The strips were supported horizontally in an apparatus which was a

modification of that of Durrum (1950), and a voltage of ~~15~~ 9 volts/cm. width applied for 3 hours, during which time the current rose from 1.0-1.3 m.amp., depending on the buffer, to 1.5-2.0 m.amp./cm. width. After drying of the strips the positions of the complexes were revealed by spraying with aniline hydrogen phthalate in glacial acetic acid followed by heating at 100° for 5 minutes; due to the presence of traces of borate buffer on the paper use of the triphenyltetrazolium chloride reagent for this purpose was shown not to be satisfactory. The position of creatinine was revealed with alkaline picrate.

In borate buffers of pH 8.0 and 8.2 resolution of a mixture of D-glucosone, D-glucose, and D-fructose was conveniently effected. The results are recorded in Table 5.; as an index of migration of the borate ion-carbohydrate complexes the term "M_o value" is introduced where for any substance

$$M_o = \frac{\text{True distance of migration of the substance}}{\text{True distance of migration of D-glucosone}}$$

Such an index, which is analogous to the M_g term of Foster (1952) in which migration of any sugar is related to that of D-glucose, is used because, under conditions of pH for which resolution of mixtures containing D-glucosone may be achieved, the borate ion-osone complex possesses the greatest mobility.

Table 5.

Sugar	M_o Values			
	pH 8.0	pH 8.2	pH 8.4	pH 8.6
D-Glucosone	1.00	1.00	1.00	1.00
D-Glucose	0.64	0.62	1.39	2.00
D-Fructose	0.94	0.91	1.51	1.91
D-Glucosone	1.00	1.00	} 0.65	} 0.82
+				
D-Glucose	0.63	0.62		

Above pH 8.4 the mobility of the osone complex decreased rapidly with increase in pH; in contrast, the mobilities of the complexes of glucose and fructose increased with increase in pH, an observation also made by Consden & Stanier (1952) with regard to these and other monosaccharides. Such results suggest the formation of a different species of complex between the osone and the borate ion at higher pH levels. In addition, resolution of mixtures of D-glucosone and D-glucose or of D-glucosone and D-fructose at pH values of 8.4 and higher did not take place owing to the apparent formation of a complex involving the osone and the other sugar with the same borate ion; single bands of low mobility were rendered visible by spraying ionophoretograms of these mixtures with the aniline hydrogen phthalate reagent (see Table 5. for results obtained for the glucosone-glucose mixture).

Separation of the pure osone and the "polymer" present in concentrates of solutions of the pure osone, as was possible on the paper chromatogram, was not achieved by ionophoresis in borate buffers at various pH levels; single bands were obtained with all specimens of the osone examined. Such observations may be considered to give support to the proposal that the second component produced during concentration is indeed a polymer; it is suggested that, in the alkaline buffers used in the ionophoresis, this polymer depolymerises to give the free osone, thus explaining why the two components apparently react with borate ions to form the same complex. The alternative explanation of the ionophoretic results, namely, that the borate ion-osone complex and the borate ion-"polymer" complex possess the same mobility even at different pH values, is unlikely.

The possibility that free glucosone contains a hydrated carbonyl group possessing an incipiently ionic hydrogen atom, as was proposed by Stacey & Turton (1946) for tetra-O-acetyl D-glucosone hydrate, suggested that migration of the osone

might occur in buffer systems not containing borate ions. Such mobility would be in contrast to the normal sugars (Consden & Stanier, 1952), although Evans, Nicoll, Strause & Waring (1928) have presented evidence that glucose and fructose in alkaline media behave as very weak acids. However, it has been shown that no migration of D-glucosone (or D-glucose or D-fructose) occurs in either phosphate-citrate buffer of pH 7.0 or in barbiturate buffer of pH 8.6 under the experimental conditions described for the borate buffers.

2.2. Chemical Properties and Reactions.

2.2.1. Oxidation of Glucosone.

Glucosone has been shown not to decolorise cold neutral permanganate solution, thus indicating the absence of either a furanose ring structure or an enediolic system in the molecule.

A study of the oxidation of D-glucosone by the method of Nelson (1944), using the improved copper reagent of Somogyi (1945), has been made; the reducing powers of various samples of the osone have been compared with that of D-glucose. The results are discussed under the heading "Methods of Estimation of Glucosone", Part II, 2.2.5.

2.2.2. Action of Acids on Glucosone.

The observation of Fischer (1889) that the action of hot dilute mineral acid on D-glucosone results in the production of furfural and laevulinic acid has been confirmed by the present author.

3% Solutions of D-glucosone, D-fructose, and D-xylose in 1N-hydrochloric acid were heated in sealed tubes at 100° for 2 hours. The solutions were then filtered from humin material, neutralised and analysed on the paper chromatogram using the upper phase of a n-butanol-acetic acid-water (4:1:5) mixture as developer and a solution of resorcinol in 2N-hydrochloric acid as identification reagent; a similar procedure was used by Gottschalk (1952) for the detection of 5-hydroxymethylfurfural formed by acid decomposition of N-substituted isoglucosamines. By this method formation of furfural from D-glucosone and D-xylose and of 5-hydroxymethylfurfural from D-fructose was demonstrated. Similar treatment of a solution of the osone in water and in 2N-acetic acid was shown not to produce furfural although some decomposition with the formation of small amounts of humin material was observed; such decomposition was not observed for D-xylose and D-fructose. Longer treatment of the

the osone with dilute hydrochloric acid gave laevulinic acid, identified on the chromatogram using triphenyltetrazolium chloride; on heating at 100° for 3 minutes the laevulinic acid was revealed as a pale blue ellipse on a pink ground. Further confirmation of the production of furfural from the osone was supplied by application of the test of Fearon (1950); the osone was heated with hydrochloric acid in the presence of an excess of urea with the production of a yellow colour, characteristic of similar treatment of pentoses. Under these conditions the hexoses, the typical acid decomposition product of which is 5-hydroxymethylfurfural, give a blue colour.

It is considered that the formation of furfural and of laevulinic acid, normally obtained by the further action of mineral acid on 5-hydroxymethylfurfural, from D-glucosone take place by two different mechanisms. Isbell (1944) has postulated that one of the stages in the production of laevulinic acid from 5-hydroxymethylfurfural is elimination of the aldehyde group of an osone-like intermediate as formic acid (see Part I, 1.5.3.); similar decomposition of D-glucosone, reacting in the open chain form, would give the pentose D-arabinose which, under the action of dilute mineral acid, would yield furfural. Preliminary oxidation of the osone to 2-oxo-D-gluconic acid, a compound which has been shown to give furfural (Ehrlich & Guttmann, 1934; Young & Rice, 1946), would provide an alternative mechanism. The production of laevulinic acid from D-glucosone without the intermediary formation of 5-hydroxymethylfurfural is more difficult to explain. Levene & Mori (1929) have shown acid decomposition of 2-deoxypentoses to yield laevulinic acid, a reaction for which Isbell (1944) has proposed a theoretical mechanism; it might be suggested that a 2-deoxypentose, or a similar compound, is one of the initial products of the action of acid on the osone, further action of the acid leading to the formation of laevulinic acid. No experimental evidence supporting these proposed mechanisms has

been obtained.

Certain comparisons may be made between the action of dilute acid on D-glucosone and on 2-oxo-D-gluconic acid. They both yield furfural, while glucosone, but not the 2-oxo aldonic acid, also gives laevulinic acid. Mild treatment with acid or even the heating of aqueous solutions of 2-oxo-D-gluconic acid causes enolisation and simultaneous lactonisation with the production of D-araboascorbic acid; this same product is also obtained by mild treatment with alkali. However, no evidence has been obtained for the formation of kojic acid, the product of reaction with dilute alkali, by heating acid or neutral solutions of D-glucosone.

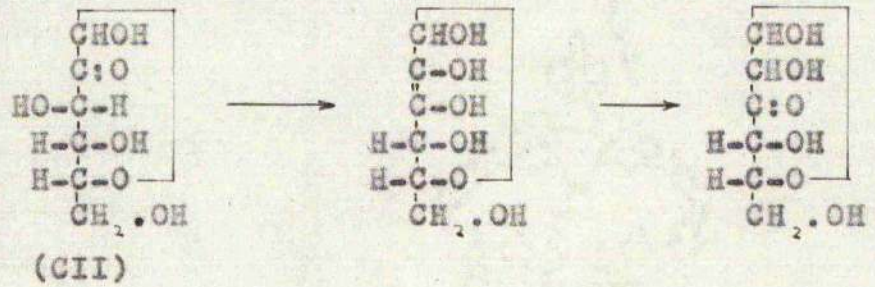
The report of Hynd (1927a) that glucosone does not give a positive reaction on application of Seliwanoff's test has been confirmed. In contrast to the results of Zerban & Sattler (1950), it has been shown that glucosone, like many carbohydrates, gives a deep green colour with the acid anthrone reagent of Dreywood (1946); 2-oxo-D-gluconic acid has also been shown to give this result.

2.2.3. Action of Alkalis on Glucosone.

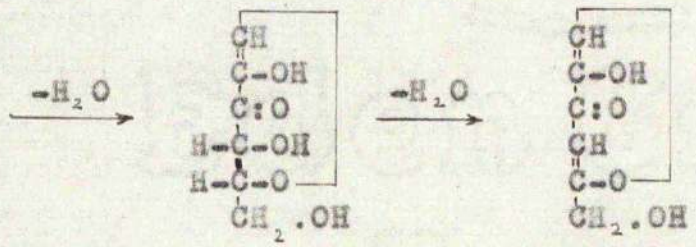
It has been shown that D-glucosone in the presence of a variety of alkaline reagents forms a product giving qualitative tests for kojic acid. Thus, a solution of the osone in pyridine, pyridine-acetic anhydride (1:1), 0.1N-sodium hydroxide, 0.1N-ammonium hydroxide, or collidine saturated with water gave a cherry-red colour on addition of a few drops of ferric chloride solution. Spectrophotometric evidence of the formation of kojic acid from the osone by the action of dilute alkali has been presented previously (see Part II, 2.1.5.).

It is interesting to compare the action of dilute alkali on D-glucosone with similar treatment of 2-oxo-D-gluconic acid. In the case of the osone (CII) the stable end-product

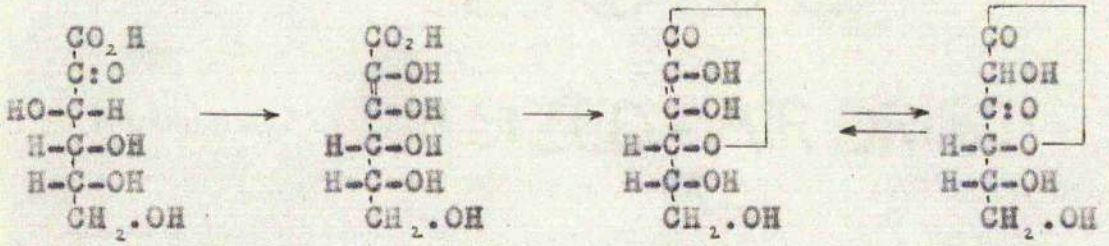
may be considered to be the γ-pyrone kojic acid (LXXVII), while for the 2-oxo acid (CIII) the product is D-araboascorbic acid (CIV) (Maurer & Schiedt, 1933; Baird, Haworth, Herbert, Hirst, Smith & Stacey, 1934), in equilibrium with 3-oxo lactone (CV), an "α,γ-dipyrone". The following general mechanisms illustrate the similarity of the two types of rearrangement:



(CII)



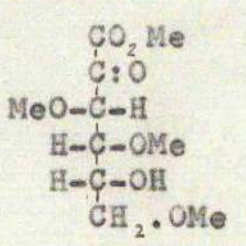
(LXXVII)



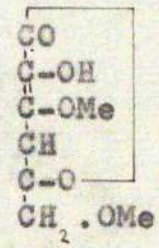
(CIII) (CIV) (CV)

During the simultaneous enolisation and lactonisation of 2-oxo-D-gluconic acid, with the formation of D-araboascorbic acid containing a γ-lactone ring, there is no loss of the elements of a molecule of water between C_4 and C_5 as is the case in the formation of kojic acid from D-glucosone. However, when formation of a γ-lactone is prevented by substitution of the hydroxyl on C_4 of the 2-oxo acid but formation of a δ-lactone is possible the similarity between the reactions of the osone and the acid towards alkaline reagents becomes more obvious. Thus, Haworth, Hirst & Jones (1938) showed that when

methyl 3:4:6-tri-O-methyl-2-oxo-D-gluconate (CVI) was treated with sodium methoxide in methanol lactonisation was brought about between C_1 and C_5; at the same time enolisation occurred together with the elimination of the elements of a molecule of methanol between C_4 and C_5 with the production of the six-membered ring analogue of ascorbic acid (CVII).



(CVI)



(CVII)

In the formation of these analogues of ascorbic acid elimination of the elements of a molecule of water between C_1 and C_2, as occurs in the transformation of D-glucosone into kojic acid, is precluded by the presence of the carboxyl group at C_1.

2.2.4. Nitrogenous Derivatives of Glucosone.

Attempts to prepare a crystalline semicarbazone or oxime from D-glucosone met with no success; these results support the proposal that the osone exists in a form containing a lactol ring arising on the potential aldehyde group at C_1 and a hydrated carbonyl group at C_2 (see Part II, 4.). No report appears in the literature of a semicarbazone or an oxime of 2-oxo-D-gluconic acid.

Analytically pure D-glucosone methylphenylhydrazone was prepared according to the method of Fischer (1889); the derivative, which reduced Fehling's solution, was shown not to give a blue colour with the arsenophosphotungstic acid reagent in the presence of alkali-cyanide and it is therefore considered that the substituted hydrazine residue is attached to C_2. The hydrazone did not regenerate the colour of Schiff's reagent, thus indicating the absence of a free aldehyde group; analytical results showed the crystalline derivative not to be

hydrated thereby suggesting that the potential aldehyde group at C_1 is involved in a lactol ring. However, it was shown that the compound did not apparently exhibit mutarotation; it is possible that mutarotation is so rapid as to escape detection.

Subsequently Ohle, Henseke & Czyzewski (1953), who reported similar properties (with the exception of the examination of the reaction with the arsenophosphotungstic acid reagent) for this hydrazone, claimed to identify it with the "β-fructose-methylphenylhydrazone" of Percival & Percival (1937). They obtained the derivative by treatment of the methylphenylosazone of D-fructose with nitrous acid as well as from D-glucosone by the method of Fischer (1889), and showed that by either method the product was identical with that of the treatment of D-fructose with the calculated amount of methylphenylhydrazine in acetic acid.

It has been demonstrated by the present author that decomposition of this methylphenylhydrazone with hydrochloric acid or benzaldehyde leads to the formation of D-glucosone.

Mandl & Neuberg (1952) described the characterisation of a number of monosaccharides as the corresponding 2:5-dichlorophenylhydrazones by reaction of the sugars with the free base in methanol. It was shown by the present author that D-glucosone reacted with 2:5-dichlorophenylhydrazine with the immediate precipitation of D-glucose 2:5-dichlorophenylosazone under the conditions described by Mandl & Neuberg (1952); D-glucose and D-fructose, even in the presence of an excess of the base, gave only the corresponding 2:5-dichlorophenylhydrazones. Thus, a means of identification and characterisation of the osone when in admixture with other sugars is provided, the dichlorophenylosazone being readily separated from the dichlorophenylhydrazones by fractional crystallisation

The ready formation of the sparingly soluble

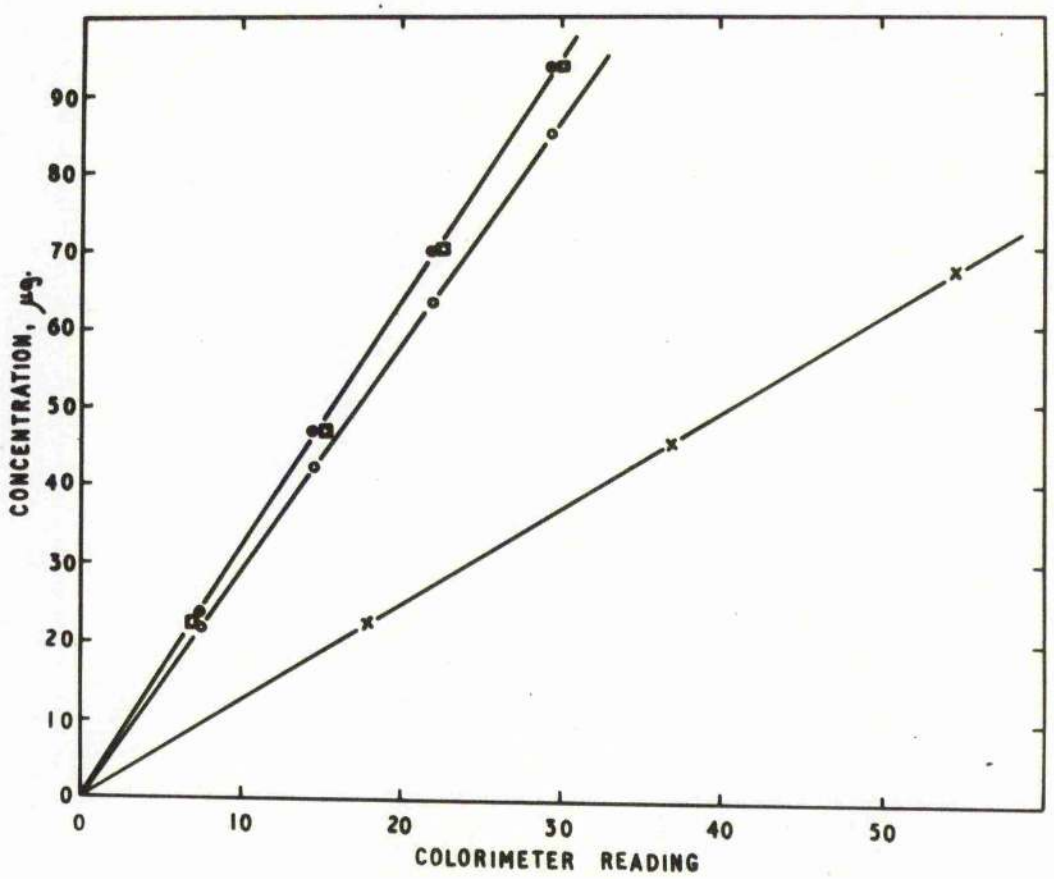
2:4-dinitrophenylosazone of D-glucose from D-glucosone has been used as the basis of a gravimetric method of estimation for various samples of the osone (see Part II, 2.2.5).

2.2.5. Methods of Estimation of Glucosone.

The osone in solution was determined gravimetrically as D-glucose 2:4-dinitrophenylosazone by reaction at 40° for 3 hours with a 1.5% solution of 2:4-dinitrophenylhydrazine in 2N-hydrochloric acid. An acid hydrolysate of tri-O-isopropylidene D-glucosone hydrate gave 96% of the theoretical yield of the dinitrophenylosazone; a standard solution of the dried osone "froth", shown chromatographically to contain both pure osone and "polymer", gave an 81% yield of the dinitrophenylosazone, calculated on D-glucosone monohydrate. It is considered that the "polymer", which was shown to be stable towards dilute mineral acid, does not form an osazone, a proposal confirmed by attempts to prepare such a derivative from solutions of the pure "polymer", isolated chromatographically. This method of estimation of the osone is of little value when other sugars are present, considerable interference occurring under the conditions described.

Comparison of the reducing powers of solutions of various samples of the osone were made with that of D-glucose by the colorimetric technique of Nelson (1944) using the improved copper reagent of Somogyi (1945). The results are recorded graphically in Fig. 4.; D-glucosone is shown to possess approximately 40% of the reducing power of D-glucose. From the graph it may be seen that the reducing power of a standard solution of the "froth" is identical with that of a hydrolysate of the crystalline tri-O-isopropylidene derivative of the osone when the product of such hydrolysis is calculated as a monohydrate. It is considered that under the influence of the alkaline conditions of the reaction the "polymer" present in solutions of the "froth" is rapidly depolymerised

Fig. 4. Determination of the Reducing Power of D-Glucosone by Nelson's Method.



◘ : D-Glucosone "froth".

● : D-Glucosone, prepared by acid hydrolysis of tri-O-isopropylidene D-glucosone hydrate, calculated as $C_6H_{10}O_6 \cdot H_2O$.

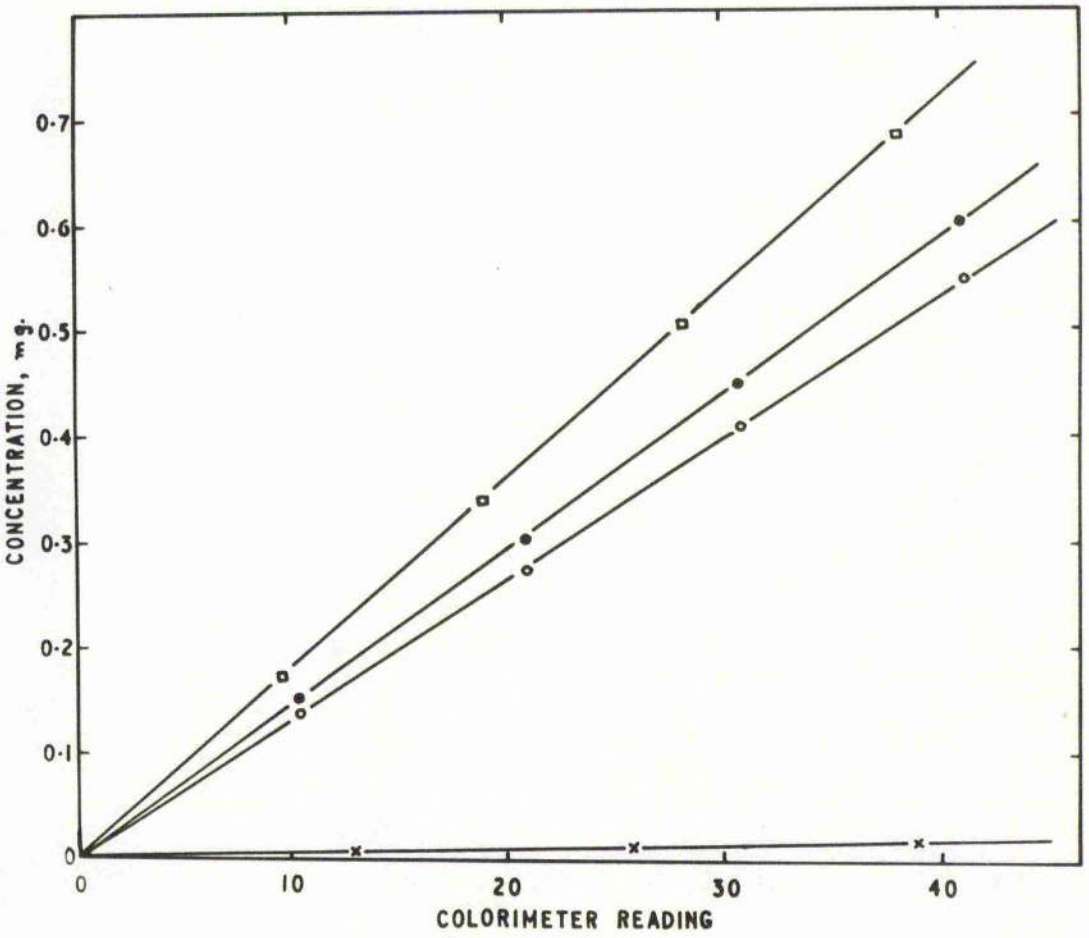
o : D-Glucosone, prepared by acid hydrolysis of tri-O-isopropylidene D-glucosone hydrate, calculated as $C_6H_{10}O_6$.

✖ : D-Glucose.

with consequent formation of the pure osone; depolymerisation in alkaline media of the otherwise stable "polymer" has been postulated previously to explain the production of ionophoretograms indicating homogeneity of solutions of concentrates of the osone (see Part II, 2.1.7.).

A colorimetric method of estimation of D-glucosone based on the reduction of Benedict's arsenophosphotungstic acid reagent for uric acid in the presence of alkali-cyanide has been evolved by the present author. The reducing power of a standard solution of the "froth" was compared with that of a hydrolysate of tri-O-isopropylidene D-glucosone hydrate and that of a standard solution of uric acid; the results are recorded graphically in Fig. 5. It may be observed that the reducing power of the hydrolysate, even when calculated on the basis of the product being D-glucosone monohydrate, is condiderably greater than that of the "froth"; it is suggested that under the short and mild alkaline conditions of the reaction depolymerisation of the "polymer" present in the solution of the "froth" does not occur. It has been shown that solutions of the pure "polymer" react only very slowly with the arsenophosphotungstic acid reagent and do not reduce Fehling's solution at room temperature. It is interesting to notice that for this method the "froth" possesses 83% of the reducing power of D-glucosone monohydrate prepared by hydrolysis of the isopropylidene derivative, while the "froth" gives 84% of the yield of D-glucose 2:4-dinitrophenylosazone given by the hydrolysate. It was shown that under the conditions of reaction of this colorimetric estimation of the osone the presence of fructose in concentrations of up to 1% did not interfere appreciably, while no colour was given on addition of the reagent to solutions of glucose; thus by use of this delicate reaction the osone in dilute solution may be both detected and estimated when in admixture with other sugars. Substances which

Fig. 5. Estimation of D-Glucosone with the Uric Acid Reagent.



◘ : D-Glucosone "froth".

● : D-Glucosone, prepared by acid hydrolysis of tri-O-isopropylidene D-glucosone hydrate, calculated as $C_6H_{10}O_6.H_2O$.

○ : D-Glucosone, prepared by acid hydrolysis of tri-O-isopropylidene D-glucosone hydrate, calculated as $C_6H_{10}O_6$.

✖ : Uric acid.

interfere with the reaction include glyoxal, pyruvic acid, 2-oxo-aldonic acids, and, of course, uric acid; thus the method is not directly applicable to the estimation of glucosone in biological material.

---

3. DERIVATIVES OF OSONES.

> "The goal of most chemical reactions in the sugar group is the obtaining of a desired sugar or some derivative of it in pure crystalline state."
>
> C. S. Hudson, 1951.

3.1. Introduction.

The lack of crystallinity of the osones makes the preparation of crystalline derivatives from which the osones may be readily regenerated in pure state a necessity; it is only by way of such derivatives that samples of the osones suitable for structural and biological investigations may be obtained. No such derivatives are described in the literature; in consequence, attempts have been made, attended by some degree of success, by the present author to make good this deficiency in the field of osone chemistry.

Attempts to prepare crystalline "glucosonides" by the established methods for the preparation of glycosides met with little success. Chromatographic evidence of the formation of an unstable, non-crystalline "glucosonide" by treatment of D-glucosone with methanol containing dry hydrogen chloride was obtained; paper chromatographic analysis of the reaction mixture using a neutral developer showed the production of a derivative possessing an R_F value greater than that of glucosone. Similar analysis employing an acid developer, namely, the upper phase of a n-butanol-acetic acid-water (4:1:5) mixture, revealed the presence of glucosone only. No "glucosonide" has been isolated, possibly due the instability of such derivatives.

Attempted methylation of D-glucosone using methyl sulphate and sodium hydroxide in the manner employed for the preparation of fully methylated derivatives from the free sugars led to decomposition of the osone under the influence of the alkali. Replacement of the alkali in this mode of alkylation

with a strong base ion-exchange resin was shown not to be effective for the methylation of fructose and in consequence application of this modification to the methylation of glucosone was not attempted. Hirst (personal communication) has suggested that sodium salts of the sugars are intermediates in the reaction thereby precluding replacement of the inorganic base with an insoluble resin.

Acetylation of glucosone with hot acetic anhydride in the presence of anhydrous sodium acetate led to the production of di-O-acetyl kojic acid and not an acetate of the osone. Maurer and his coworkers showed that crystalline acetates of glucosone, prepared by indirect synthesis, are also converted into the kojic acid derivative on attempted further acetylation. It is therefore possible that treatment of the free osone as described above does yield an acetylated derivative of the sugar which, in the presence of an excess of the reagents, is immediately transformed into the diacetate of the γ-pyrone.

The desired crystalline derivatives of the osones, from which the pure sugars may be readily regenerated, have been obtained by condensation with acetone; the preparation, properties, and structures of a number of isopropylidene derivatives of a variety of osones are described in the following pages.

3.2. isoPropylidene Derivatives of Osones.

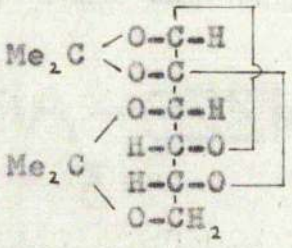
3.2.1. Introduction.

The condensation of carbohydrates with acetone has long been accepted as a method for the preparation of readily crystallisable derivatives, which may be employed for the investigation of the structure of the original sugar as well as providing valuable intermediates for synthetic studies; in addition, isopropylidene derivatives, being easily hydrolysed by dilute mineral acids, provide a means of preparation of the pure sugar. In the case of the osones the preparation of such derivatives has not been previously exploited; a full investigation has been made by the present author of the isopropylidene derivatives of a variety of osones, preliminary results of which have been published (Bayne, Collie & Fewster, 1952).

3.2.2. D-Glucosone.

3.2.2.1. Tri-O-isoPropylidene D-Glucosone Hydrate.

Collie (1941) reported the preparation of a crystalline derivative by the treatment of D-glucosone with anhydrous acetone containing concentrated sulphuric acid as catalyst. In spite of analytical difficulties he claimed to show that the derivative contained two isopropylidene groups, and, mainly on evidence of a theoretical nature, formulated it as 1:2-3:6-di-O-isopropylidene D-glucosone (CVIII), containing 1:4- and 2:5-furanose rings.



(CVIII)

Collie was unable to achieve partial hydrolysis of the compound although he demonstrated that complete hydrolysis, either with dilute mineral acid or boiling water, produced free glucosone, identified qualitatively.

The present author showed that when D-glucosone, in the form of a "froth", was shaken with anhydrous acetone containing 4% (v/v) of concentrated sulphuric acid it dissolved fairly rapidly; such a high concentration of acid was first used by Ohle & Koller (1924) for the preparation of di-O-isopropylidene glucose. From a solution of the product of condensation, isolated in the usual manner, in methanol or dioxan a crystalline derivative, showing physical properties similar to those reported by Collie (1941), was obtained in rather low yield. From the results of elementary analysis and of isopropylidene group determination this derivative was considered to be tri-O-isopropylidene D-glucosone hydrate. The derivative did not reduce Fehling's solution, even on boiling, and no colour was formed with the arsenophosphotungstic acid reagent in the presence of alkali-cyanide. The failure to prepare methyl ethers or acetyl esters under fairly drastic conditions established that substitution was complete; such a conclusion was supported by the observation that the osone derivative, unlike the di-O-isopropylidene derivatives of the hexoses, which contain one free hydroxyl group, was insoluble in water. On complete hydrolysis, either with 0.1N-mineral acid or 85% acetic acid, D-glucosone, characterised and estimated as D-glucose 2:4-dinitrophenylosazone, was obtained almost quantitatively; D-glucosone could not be identified chromatographically after boiling a suspension of the derivative in water although complete hydrolysis was shown to occur on boiling in the presence of cation-exchange resin. Since after short exposure of the isopropylidene derivative to cold 0.1N-sulphuric acid the crystalline compound was not recoverable it was presumed that at least one of the isopropylidene groups was labile; it has been pointed out by Bell (1947) that such behaviour is characteristic of those fully-substituted isopropylidene derivatives of sugars which possess a furanose ring, an exception existing in 1:2-4:5-di-O-isopropylidene fructose which, although

partially hydrolysed by cold dilute acid (Irvine & Garrett, 1910), is not considered to be a fructofuranose structure. Bell (1947) has also shown the labile isopropylidene group to be attached generally to the primary alcoholic group of the sugar.

By the method described crystalline tri-O-isopropylidene D-glucosone hydrate was obtained in yields of 12-15%; these yields were supplemented to a small extent by chromatographic development of the non-crystalline residues on an alumina column with benzene-ether mixtures. Two further fractions were obtained from the column, one of which was identified as di-O-isopropylidene D-glucosone hydrate, characterised as its diacetate (see Part II, 3.2.2.2.); final elution of the column with methanol gave a small amount of free D-glucosone. Alternatively, further treatment of the non-crystalline residues with acetone-sulphuric acid gave the tri-O-isopropylidene derivative.

Alternative methods of condensation of glucosone with acetone were investigated in the hope of improving the yield of crystalline derivative. The presence of anhydrous copper sulphate in the reaction medium was shown not to increase the yield. Evaporation of an aqueous solution of the osone in the presence of kieselguhr to form a solid mass which was powdered and dried *in vacuo* and then treated with acetone containing sulphuric acid, according to the method of Bacon & Bell (1948) for the preparation of 2:3-4:5-di-O-isopropylidene D-fructose from fructose in foetal blood, gave the tri-O-isopropylidene derivative in 12% yield. The use of glucosone in the form of a white amorphous powder, obtained by addition of ether to an ethanolic solution of the osone, for the condensation facilitated solution but did not lead to an increase in the yield of the derivative. Application of the procedure of Grunenberg, Bredt & Freudenberg (1938) using fused zinc chloride as catalyst and a mixture of phosphorus pentoxide and

syrupy orthophosphoric acid as dehydrant, gave 10-12% yields of the derivative; Grunenberg *et al.* (1938) claimed to obtain high yields of acetone sugars by their method and have pointed out that, "due possibly to the acidic acetonisation medium the same acetone products are obtained which are formed by the use of sulphuric acid as catalyst". Treatment of a solution of glucosone in glacial acetic acid with dry acetone containing anhydrous zinc chloride was attempted but removal of excess zinc chloride with hydrogen sulphide after neutralisation caused degradation of the reaction products. Low yields of the tri-*O*-*iso*propylidene derivative were obtained by shaking glucosone "froth" with a suspension of cation-exchange resin in dry acetone at room temperature for 5 days; such a method has the advantage over those involving the use of high concentrations of acid of the absence of side products such as mesityl oxide and mesitylene, as well as requiring no neutralisation. This mode of condensation has been utilised recently by Wadman (1952) for the preparation of di-*O*-*iso*propylidene D-glucose and *iso*-propylidene derivatives of mannitol.

The only other example of a tri-*O*-*iso*propylidene derivative of a sugar structure containing six carbon atoms, other than the hexitols, is the tri-*O*-*iso*propylidene D-gluconic acid of Haworth, Hirst & Jones (1937). Like the tri-*O*-*iso*propylidene derivative of glucosone this derivative was obtained, from crystalline calcium gluconate, in comparatively low yield. The explanation of the widely varying yields of the products of the condensation of sugars and sugar derivatives with carbonyl compounds is not obvious; thus, a tri-*O*-ethylidene derivative may be obtained from mannitol in 95% yield (Meunier, 1891), while a corresponding derivative of sorbitol, prepared under similar conditions, is obtained in 18% yield (Sullivan, 1945; Bourne & Wiggins, 1948). The possibility that other isomeric forms, which may not necessarily be readily crystallised, may be formed simultaneously cannot be excluded; thus, two isomers of

tri-O-ethylidene sorbitol have been reported (Sullivan, 1945), while similar types of isomerism have been described by Haskins, Hann & Hudson (1942) and Hann & Hudson (1944) in the hexitol series. Bell (1936) reported the simultaneous formation of two isomeric mono-O-isopropylidene derivatives of methyl 2-O-methyl-D-glucofuranoside, while Oldham & Honeyman (1946) showed that methyl-β-L-arabinoside gave a mixture of two 3:4-O-benzylidene derivatives, only one of which was obtained crystalline; a similar pair of ethylidene derivatives was also obtained. At the same time the nature of the condensation product derived from any given pair of reactants is not always independent of the type of catalyst used, particularly with regard to the degree of condensation. Thus, the low yield of crystalline tri-O-isopropylidene D-glucosone hydrate obtained by the original method described above may be explained on the following grounds: a non-crystalline isomeric tri-O-isopropylidene derivative may be formed simultaneously, although such a derivative was not isolated from the non-crystallisable residues by chromatographic analysis; the conditions of condensation may not have been sufficiently rigorous to bring about complete substitution of all the substrate, since in addition to the crystalline tri-O-isopropylidene derivative a non-crystalline di-O-isopropylidene derivative was obtained in good yield and was shown to be identical with the product of partial hydrolysis of the completely substituted derivative (see Part II, 3.2.2.2.).

3.2.2.2. Di-O-isopropylidene D-Glucosone Hydrate.

When tri-O-isopropylidene D-glucosone hydrate was dissolved in 85% acetic acid (in which it is more soluble than in aqueous methanol or aqueous acetone) and maintained at 50° it was apparent that slow hydrolysis took place; the hydrolysis could be followed polarimetrically in spite of the low specific rotation of the starting material. A break in the fall in

Fig. 6. Hydrolysis of Tri-O-isoPropylidene D-Glucosone Hydrate (c, 4.80 in 85% acetic acid).

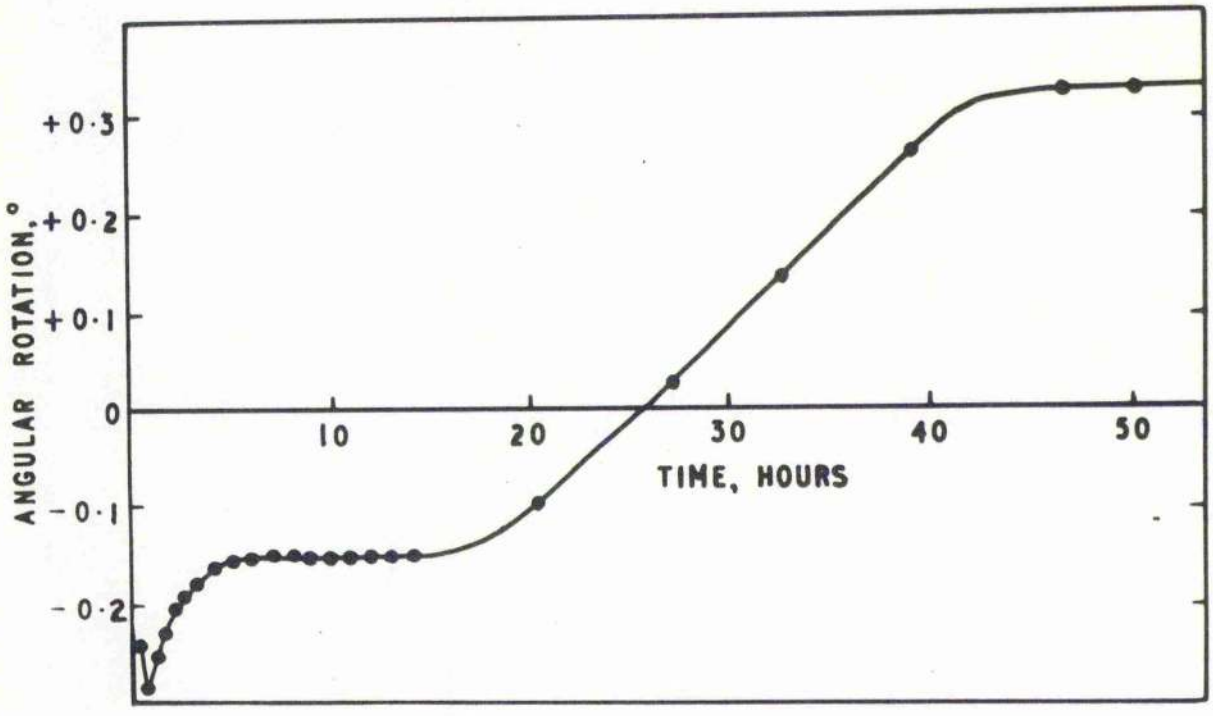
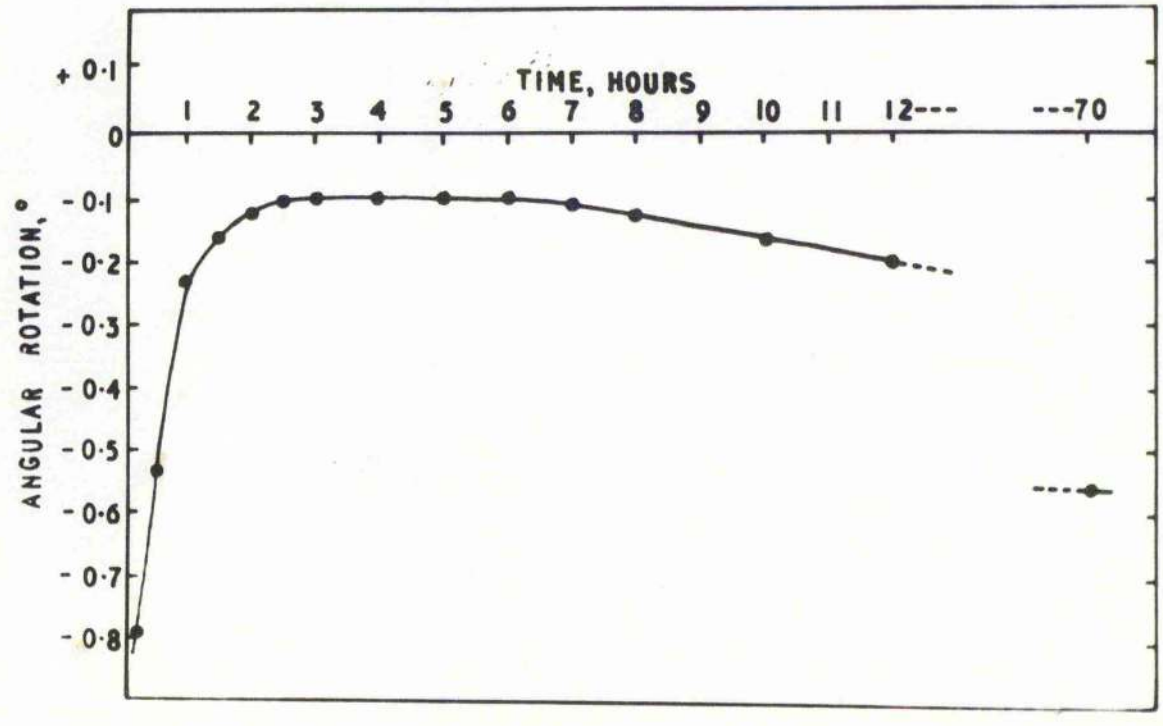


Fig. 7. Hydrolysis of Tri-O-isoPropylidene L-Gulosone Hydrate (c, 5.00 in 85% acetic acid).



rotation after 5 hours suggested the completion of one stage of hydrolysis (see Fig. 6.); at this point the solvent was evaporated and a non-reducing syrup was obtained which could not be crystallised. Acetylation of this product with acetic anhydride in the presence of anhydrous sodium acetate gave a crystalline derivative; from the results of elementary analysis, isopropylidene group determination, and estimation of acetyl groups by direct titration the crystalline derivative was concluded to be di-O-acetyl-di-O-isopropylidene D-glucosone hydrate.

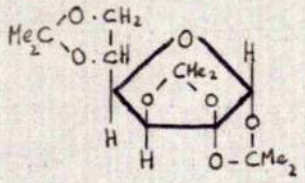
One of the non-crystalline fractions obtained in good yield, either by columnar chromatography or by chloroform extraction, from the residues of the condensation of D-glucosone with acetone was identified as di-O-isopropylidene D-glucosone hydrate, characterised as its diacetate.

3.2.2.3. Determination of the Structures of the isoPropylidene Derivatives of D-Glucosone.

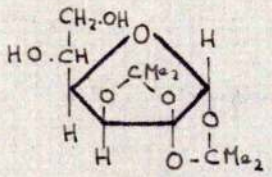
The demonstration, by direct titration, that two acetyl were present in di-O-acetyl-di-O-isopropylidene D-glucosone hydrate is important since it shows that the isopropylidene group removed was not attached to the hydrated carbonyl group at C_2 of the sugar. Stacey & Turton (1946) have shown the free hydroxyl group on this carbon atom in tetra-O-acetyl D-glucosone hydrate to possess an incipiently ionic hydrogen atom; thus, if the labile isopropylidene group in tri-O-isopropylidene D-glucosone hydrate were attached in such a position the direct titration of the diacetate of di-O-isopropylidene D-glucosone hydrate should have required three equivalents of alkali.

It was demonstrated that one molecular proportion of periodate was consumed by di-O-isopropylidene D-glucosone hydrate, prepared in solution by deacetylation of its diacetate, with the production of one molecular proportion of formaldehyde, estimated as its dimedone derivative. The di-O-isopropylidene derivative is therefore assigned the structure 1:2-2:3-di-O-

-isopropylidene D-glucosone hydrate (CX), and the parent compound that of 1:2-2:3-5:6-tri-O-isopropylidene D-glucosone hydrate (CIX), each containing a 1:4-furanose ring.



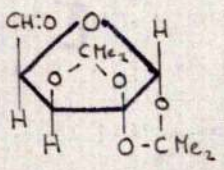
(CIX)



(CX)

These structures were confirmed by methylation, by the method of Purdie & Irvine (1903), of (CX) with the formation of di-O-methyl-di-O-isopropylidene D-glucosone hydrate; acid hydrolysis of this latter derivative gave the di-O-methyl osone which was characterised as 5:6-di-O-methyl D-glucose p-bromophenylosazone. The di-O-methyl osone was also completely oxidised with periodate with the formation of αβ-dimethoxypropaldehyde (di-O-methyl glyceraldehyde), characterised as the p-bromophenacyl derivative of αβ-dimethoxypropionic acid (di-O-methyl glyceric acid). The structure of 5:6-di-O-methyl D-glucose has been similarly confirmed by Salmon & Powell (1939).

From the products of periodate oxidation of the di-O-isopropylidene derivative after the addition of an aqueous solution of dimedone was isolated not only the bisdimedone of formaldehyde but a further crystalline fraction; the expected carbohydrate product of the oxidation is 1:2-2:3-di-O-isopropylidene 5-aldo-D-xylosone hydrate (CXI) and it is presumed that its bisdimedone is insoluble in water.



(CXI)

Bell (1948) did not observe the precipitation of carbohydrate bisdimedones following periodate oxidation of partially methylated sugars, and periodate oxidation of 1:2-O-isopropylidene D-glucose, under the same conditions, yielded only the dimedone derivative of formaldehyde, the oxidation product, 1:2-O-isopropylidene 5-aldo-D-xylose (Iwadare, 1941), remaining in solution, presumably as its bisdimedone. However, Bourne, McSweeney & Wiggins (1952) have shown the bisdimedone of the 2:3-4:5-di-O-isopropylidene derivative of aldehydo-L-xylose to be insoluble in water; such a compound would be expected to exhibit solubility properties similar to those of the bisdimedone of the osone fragment described above.

3.2.3. L-Glucosone.

Crystalline tri-O-isopropylidene L-glucosone hydrate was obtained by treatment of L-glucosone with acetone containing concentrated sulphuric acid in the manner described initially for the preparation of the corresponding derivative of D-glucosone.

3.2.4. 3-O-Methyl D-Glucosone.

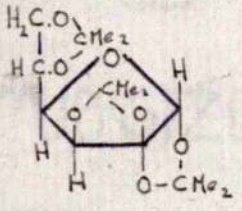
Treatment of 3-O-methyl D-glucosone with acetone and sulphuric acid gave a product, isolated in the usual manner, which could not be crystallised. The derivative was non-reducing, was insoluble in water, and gave qualitative tests for an isopropylidene derivative; decomposition occurred on attempted distillation in vacuo, and on hydrolysis with dilute mineral acid 3-O-methyl D-glucosone, characterised as 3-O-methyl D-glucose phenylosazone, was obtained.

3.2.5. L-Gulosone.

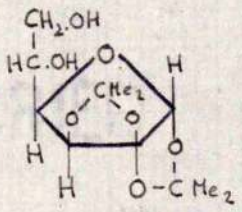
Condensation of L-gulosone with acetone in the manner described for D-glucosone gave a crystalline derivative in 15% yield which was identified analytically as a tri-O-isopropylidene L-gulosone hydrate. The compound was shown to possess physical properties similar to those of the corresponding D-glucosone

derivative; the L-gulosone derivative was shown to be non-reducing and to be completely substituted. Acid hydrolysis gave L-gulosone, characterised as L-gulose phenylosazone. One of the isopropylidene groups was shown to be labile, suggesting the presence of a furanose ring structure as was shown to be present in tri-O-isopropylidene D-glucosone hydrate. Hydrolysis of the L-gulosone derivative in 85% acetic acid could be followed polarimetrically (see Fig. 7.) and a break in the fall in rotation after 3 hours suggested the completion of one stage of hydrolysis; at this point a non-reducing syrup was isolated which could not be crystallised but on acetylation a crystalline derivative was obtained and identified analytically as di-O-acetyl-di-O-isopropylidene L-gulosone hydrate; it showed physical and chemical properties similar to those of the diacetate of di-O-isopropylidene D-glucosone hydrate.

The presence of a 1:4 furanose ring in the isopropylidene derivatives of L-gulosone was confirmed by observation of the consumption of one molecular proportion of periodate with the simultaneous production of one molecular proportion of formaldehyde by di-O-isopropylidene L-gulosone hydrate, prepared in solution by deacetylation of its crystalline diacetate. Hence, the di-O-isopropylidene derivative is formulated as 1:2-2:3--di-O-isopropylidene L-gulosone hydrate (CXIII) and the parent compound as 1:2-2:3-5:6-tri-O-isopropylidene L-gulosone hydrate (CXII).



(CXII)



(CXIII)

From the dimedone derivatives of the products of periodate oxidation, as well as the bisdimedone of formaldehyde a crystalline derivative was isolated which was shown to be identical

with that obtained from similar oxidation of di-O-isopropylidene D-glucosone hydrate, and identified tentatively as the bis-dimedone of 1:2-2:3-di-O-isopropylidene 5-aldo-D-xylosone hydrate (CXI). The isolation of such a derivative confirms the structures assigned to the isopropylidene derivatives of both D-glucosone and L-gulosone, a pair of sugars whose only configurational difference exists at C_5, the asymmetry of which carbon atom is destroyed by periodate oxidation of the corresponding di-O-isopropylidene derivatives with the formation of a common product only if the derivatives possess the proposed structures.

3.2.6. D-Xylosone.

Condensation of D-xylosone with acetone gave a non-reducing product which could not be obtained crystalline. This product gave qualitative tests for an isopropylidene derivative and on hydrolysis with acid formed D-xylosone, characterised as D-xylose phenylosazone.

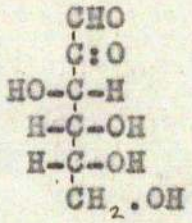
3.2.7. Conclusions.

It has been shown that condensation with acetone with the production non-reducing derivatives is a general reaction in the osone group. Tri-O-isopropylidene D- and L-glucosone hydrate and tri-O-isopropylidene L-gulosone hydrate are the first crystalline derivatives to be prepared directly from the respective osones and from which these sugars may be readily regenerated. The moderate stability of the osones towards acid minimises their decomposition when the crystalline derivatives are hydrolysed, such hydrolysis affording osones with a higher standard of purity for both structural and biological investigations than has hitherto been available. Thus, pure D-glucosone obtained in this manner has been employed for the study of the rotational, spectrophotometric, chromatographic, and ionophoretic properties of the osone; at the same time the tri-O-isopropylidene derivative has provided a standard for a number of methods of estimation of D-glucosone.

In addition to the value of these crystalline derivatives as possible synthetic intermediates and sources of the pure osones, from a study of their structures a number of conclusions may be drawn regarding the structural features of the free osones. Since the total yields of the corresponding tri- and di-O-isopropylidene osone hydrates of D- and L-glucosone and of L-gulosone are comparatively high it may be concluded that the major part of these osones condense with acetone in the form of stable hydrates, the elements of the molecule of water being an integral part of the osone structures and not merely an associated molecule of water analogous to water of crystallisation; the demonstration of these isopropylidene derivatives as hydrated structures confirms the previously recorded observation (see Part II, 2.1.2. and 2.2.5.) of the analytical behaviour of D-glucosone "froth" as a monohydrate. The formulation of a 1:4-furanose ring in the isopropylidene derivative in no way implies the presence of a similar ring in the free osones; the behaviour of D-glucosone towards oxidising agents such as hypoiodite (Myrback, 1939) and lead tetra-acetate (Becker & May, 1949) suggests the presence of a 1:5-pyranose ring in the molecule. Under the influence of the acid conditions of condensation conversion to a furanose form is considered to occur; a similar conversion takes place during condensation of glucose with acetone.

4. THE STRUCTURE OF GLUCOSONE.

From the general reactions and properties of D-glucosone the open chain structure (LXXXI) proposed by Fischer (1889) may be accepted provisionally.



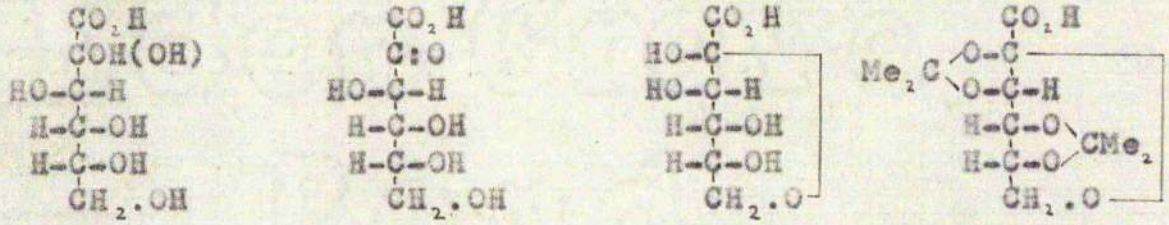
(LXXXI)

However, it has been shown by the present author from the results of elementary analysis (see Part II, 2.1.2.) that the osone, as isolated in the form of a "froth", exists as a monohydrate of molecular formula $C_6H_{12}O_7$, that is $C_6H_{10}O_6.H_2O$. Such a molecular formula is supported by the results obtained for the estimation of glucosone employing a hydrolysate of the crystalline tri-O-isopropylidene derivative as a standard (see Part II, 2.2.5. and 3.2.7.).

No evidence has been obtained by the author for the presence of a free aldehyde group in glucosone when in aqueous or alcoholic solution; thus, solutions of the osone, even after being boiled, did not regenerate the colour of Schiff's reagent and did not give an addition compound with dimedone. It may therefore be concluded that the potential aldehyde group at C_1 is either very stably hydrated or involved in a lactol ring. It is generally recognised that compounds containing a hydrated aldehyde group, although exhibiting none of the physical properties to be associated with a free aldehyde, may nevertheless behave chemically as such owing to the instability under fairly mild conditions of the hydrated group; however, it has been shown that chloral hydrate does not regenerate the colour of Schiff's reagent and does not yield an addition compound with dimedone although, on heating, it does reduce Fehling's solution. From a study of the ultraviolet absorption spectra of aqueous

solutions of glucosone (Part II, 2.1.5.) evidence of the existence of at least a proportion of the molecule being present in a form containing a free carbonyl group has been obtained. The previous evidence described above rules out the possibility that the free carbonyl group is that of a free aldehyde group and such a group is therefore to be associated with C_2; such a conclusion is supported by reports of other workers that, in neutral solution, the aldoses, in contrast to the ketoses, exhibit no selective absorption of ultraviolet light corresponding to a free carbonyl group (see Part II, 2.1.5.). The instantaneous production of a blue colour on addition of Benedict's arsenophosphotungstic acid reagent for uric acid to a solution of glucosone in the presence of alkali-cyanide also argues for the presence of a free carbonyl group; Ariyama (1927) showed glyoxal to give a similar reaction with this reagent, while the present author has demonstrated that pyruvic acid as well as 2-oxo-D-gluconic acid, compounds recognised as containing a free carbonyl group at C_2, also give the colour immediately. Fructose gives the reaction only weakly unless exposed to the prolonged action of alkali, the rate of development of colour presumably being dependent upon the rate of opening of the lactol ring on C_2 in this sugar; the initial reaction of fructose, which was shown to be very weak, may be accounted for by the presence of a small proportion of the open chain form of the sugar considered to be present in aqueous solution. The observation of Niederhoff (1929) that a solution of 2-oxo--D-gluconic acid showed no absorption of ultraviolet light corresponding to a free carbonyl group, was interpreted by this worker as indicating the presence of a lactol ring on C_2; an alternative interpretation, however, is that in such solutions the carbonyl group is hydrated, such hydration being stabilised by the presence of the adjacent free carboxyl group. This proposal of the absence of a lactol ring in the acid is supported by the observation of Niederhoff (1929) himself that the

free acid did not exhibit mutarotation. Thus, in alkaline solution the stabilising influence of the carboxyl group is decreased due to neutralisation and hence the compound gives the reaction with the arsenophosphotungstic acid reagent characteristic of a free carbonyl group; at the same time the free carbonyl group of a proportion of this open chain modification may engage in a lactol ring since Ohle & Berend (1927) showed that condensation of barium 2-oxo-D-gluconate with acetone gave the 2:3-4:5-di-O-isopropylidene derivative (CXVII); in addition, it has been shown that esters and salts of the acid exhibit mutarotation (Ohle & Berend, 1927; Ohle & Wolter, 1930). Thus, depending upon the pH of the solution, 2-oxo-D-gluconic acid may be considered to be present in solution in a number of different modifications: a hydrated open chain form (CXIV) which on addition of alkali may form the free carbonyl structure (CXV), the latter giving the blue colour with the arsenophosphotungstic acid reagent; the free carbonyl structure (CXV) may also give the modification (CXVI), containing a 2:6-fructopyranose ring, which, on condensation with acetone, yields (CXVII), and which is the structural form of the mutarotating esters and salts.



(CXIV) (CXV) (CXVI) (CXVII)

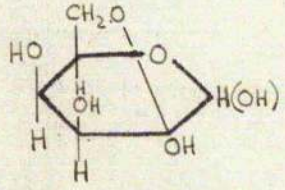
The fact that fructose gives the blue colour much less readily than does glucosone is further evidence for the absence of a lactol ring associated with C_2 in the osone. With regard to C_2 D-glucosone may be considered as being intermediate between 2-oxo-D-gluconic acid and D-fructose; in the 2-oxo-aldonic acid, owing to the proximity of the carboxyl group, the carbonyl group at C_2 is stably hydrated in acid or neutral solution; it has been shown that in the solid state the carbonyl group of glucosone is hydrated but in solution such hydration

is unstable (being less strongly stabilised by the influence of the adjacent potential aldehyde group) and a proportion of the osone exists in a form containing a free carbonyl group, thus accounting for the exhibition by solutions of the osone of selective absorption in the ultraviolet region corresponding to such a group; in fructose the carbonyl group is preferentially involved in a lactol ring while a small proportion exists in a free carbonyl form, the primary alcoholic group providing no stabilising influence for a hydrate.

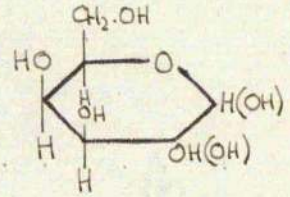
The exhibition of mutarotation by glucosone solutions suggests the presence of at least one lactol ring in the molecule. The observation by Becker & May (1949) that no formaldehyde is produced on lead tetra-acetate oxidation of the osone indicates that the hydroxyl group at C_5 is probably concerned in such a ring system; since glucosone has been shown not to decolorise cold neutral permanganate solution a furanose ring is considered to be absent, apart from the fact that the carbonyl group at C_2 is most probably present in a hydrated form. It is therefore proposed that glucosone contains a 1:5-glucopyranose ring; such a proposal is further supported by the work of Myrbäck (1939) who demonstrated that D-glucosone is oxidised by hypoiodite at approximately the same rate as is D-glucose (see Part I, 2.2.1.3.).

It has been suggested by several workers (Hynd, 1927a; Becker & May, 1949; Petuely, 1952) that glucosone may exist in a form containing two lactol rings, each to be associated with one of the two reducing centres. Such a structure would not allow of the existence of a hydrate and would not react immediately with the arsenophosphotungstic acid reagent. In addition, although the observation by Becker & May (1949) that the osone consumes three molecular proportions of lead tetra-acetate has been interpreted by them as representing the oxidation of structures such as (CXVIII) the results are open to an alternative explanation. The hydrated structure (CXIX) would

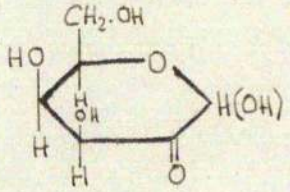
similarly consume three molecular proportions of oxidant without formation of formaldehyde; evidence that such hydration represents an integral part of the molecule is also provided by these numerical results, since oxidation of structure (CXX), containing a free carbonyl group, in glacial acetic acid, a non-hydroxylating solvent (Baer, 1940), would lead to the consumption of one molecular proportion of oxidant (see Part I, 4.).



(CXVIII)

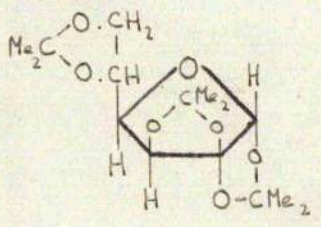


(CXIX)



(CXX)

No crystalline derivative of glucosone has been obtained in which two lactol rings are present. On the other hand, Maurer and his coworkers prepared crystalline acetates and benzoates corresponding to (CXIX) (see Part I, 1.4.1.) and these derivatives, although prepared indirectly, have been shown by the present author to be true derivatives of glucosone (see Part II, 1.4.1.); at the same time Stacey & Turton (1946) specified the possible reasons for the hydration of the carbonyl at C in these acetates. The final and most convincing evidence for the existence of glucosone as (CXIX) is the direct preparation by the present author (see Part II, 3.2.2.) of a crystalline <u>iso</u>propylidene derivative structurally identified as 1:2-2:3-5:6-tri-<u>O</u>-<u>iso</u>propylidene D-glucosone hydrate (CIX)



(CIX)

A discussion of the full significance of the structure of this derivative has been presented previously (see Part II, 3.2.7.).

Glucosone as (CXIX), capable of reacting in the free carbonyl form (CXX), may be expected to polymerise in the same manner as do other carbonyl-containing compounds of the same type such as pyruvic acid and hydroxypyruvic aldehyde. Such polymerisation would explain the complex rotational behaviour of solutions of the osone "froth" as well as the production of solutions shown, chromatographically, to be heterogeneous on concentration of solutions of the pure osone (see Part II, 2.1.4. and 2.1.6.).

PART III: A DETAILED ACCOUNT OF EXPERIMENTAL METHODS EMPLOYED BY THE AUTHOR.

CONTENTS.

Melting points are uncorrected. Microanalyses for carbon and hydrogen are by Drs. Weiler and Strauss, Oxford.

---

1. THE PREPARATION OF VARIOUS SUGAR DERIVATIVES.

1.1. 1:2-5:6-di-O-isoPropylidene D-Glucose.

a) Anhydrous D-glucose (25g.) was shaken with dry acetone (500ml.) containing concentrated sulphuric acid (15.0ml.) and anhydrous copper sulphate (50g.) for 15 hours at room temperature. After cooling the reaction mixture was neutralised with anhydrous sodium carbonate, filtered, and evaporated to a semi-crystalline syrup. Recrystallisation from methanol gave 1:2-5:6-di-O-isopropylidene D-glucose (25g., 69.0%), m.p. 110°, $[\alpha]_D^{18}$ -12.5° (c, 2.0 in chloroform).

b) By the method of Glen, Myers & Grant (1951) the derivative was obtained in 70.0% yield, m.p. 111°.

c) Condensation of D-glucose with acetone employing fused zinc chloride as catalyst and a mixture of phosphorus pentoxide and 85% phosphoric acid as dehydrant, after the manner of Grunenberg, Bredt & Freudenberg (1938), gave di-O-isopropylidene glucose in 72·0% yield, m.p. 111° after recrystallisation from methanol.

1.2. 1:2-O-isoPropylidene D-Glucose.

Di-O-isopropylidene D-glucose (40g.) was hydrolysed according to the directions of Meyer & Reichstein (1946) to give 1:2-O-isopropylidene D-glucose (30g., 88.0%), m.p. 158-160° after recrystallisation from ethyl acetate.

1.3. 2:3-4:5-Di-O-isoPropylidene D-Fructose.

a) By the method of Pacsu, Wilson & Graf (1939) (a modification of the procedure of Ohle & Koller, 1924) the derivative was

obtained in 62.0% yield, m.p. 97°, $[\alpha]_D^{18}$ -32.9° (c, 1.00 in water), after recrystallisation from 25% ethanol.

b) Hydrolysis of sucrose followed by condensation with acetone according to the method of Ohle & Wolter (1930), using concentrated sulphuric acid (4%, v/v) as hydrolysing agent and catalyst and 10N- sodium hydroxide as neutralising agent , gave di-O-isopropylidene fructose in 56.0% yield, m.p. 96° after recrystallisation from 25% ethanol.

1.4. 3-O-Methyl D-Glucose.

1:2-5:6-Di-O-isopropylidene D-glucose (75g.) was methylated by the method of Glen, Myers & Grant (1951). The syrupy product of 3-O-methyl-1:2-5:6-di-O-isopropylidene D-glucose (74g., 94.0%) was hydrolysed by boiling with an aqueous suspension of cation-exchange resin (Amberlite I.R. 120-H) (Glen, Myers & Grant, 1951) to yield 3-O-methyl D-glucose (38.6g., 75.0%), m.p. 168° after recrystallisation from methanol. (Yield of 3-O-methyl D-glucose, based on D-glucose, 48.0%).

1.5. 3:4:6-Tri-O-Methyl D-Fructose.

Tri-O-methyl inulin, prepared from inulin triacetate according to the directions of Haworth & Streight (1932), was hydrolysed by the method of Haworth, Hirst & Percival (1932); distillation of the syrupy product in a vacuum (120°/0.05mm.) gave 3:4:6-tri-O-methyl D-fructose, $[\alpha]_D^{18}$ +25.5° (c, 1.00 in chloroform).

1.6. 3:5:6-Tri-O-Methyl D-Glucose.

1:2-isoPropylidene D-glucose (40g.) in dry acetone was treated with methyl sulphate (78ml.) and powdered sodium hydroxide (60g.) for 8 hours according to the directions of Glen, Myers & Grant (1951) for the methylation of di-O-isopropylidene glucose; syrupy 3:5:6-tri-O-methyl-1:2-O-isopropylidene D-glucose (47g., 97.0%) was isolated and hydrolysed by boiling

with an aqueous suspension of cation-exchange resin (Amberlite I.R. 120-H). The syrupy product was distilled in a vacuum (130-134°/0.05mm.) to give 3:5:6-tri-O-methyl D-glucose (27.0g., 68.0%), $[\alpha]_D^{18}$ -25.8° (c, 1.00 in water).

1.7. 2:3:4:6-Tetra-O-Acetyl-2-Oxy-D-Glucal.

D-Glucose was treated with acetic anhydride, bromine, and red phosphorus according to the directions of Martos & Körösy (1950) to give 2:3:4:6-tetra-O-acetyl-D-glucosyl bromide in 93% yield, m.p. 87° after recrystallisation from ether. The acetylglucosyl halide was treated with diethylamine by the method of Maurer (1929) to give 2:3:4:6-tetra-O-acetyl-2-oxy-D-glucal in 50% yield, m.p. 63° after recrystallisation from aqueous ethanol.

1.8. N-p-Tolyl D-isoGlucosamine.

The derivative was obtained in 84% yield by the method of Weygand (1940), m.p. 153°, $[\alpha]_D^{17}$ -23.0 → -10.0° (24 hours) (c, 1.00 in methanol).

---

2. PREPARATION OF OSAZONES.

2.1. D-Glucose Phenylosazone.

a) D-Glucose hydrate (99g., 0.5 mole), freshly distilled p-toluidine (65.5g., 0.625 mole), water (75ml.), and 2N-acetic acid (25ml.) were heated together at 100° for approximately 5 minutes. A hot solution of freshly distilled phenylhydrazine (177ml., 1.66 moles) in 2N-acetic acid (1,250ml.) was added with stirring. The phenylosazone separated out in 2 minutes as pale yellow crystals. The mixture was heated for a further 30 minutes, cooled and filtered. The product was washed twice with 2N-acetic acid, three times with water and twice with ethanol-ether (2:3). After drying in a desiccator in the dark the D-glucose phenylosazone (130g., 72.5%) gave m.p. 206° (decomp.). Recrystallisation from absolute ethanol gave pale yellow crystals, m.p. 208° (decomp.), $[\alpha]_D^{18}$ -58.5 → -36° after 24 hours (c, 1.00 in pyridine-ethanol, 2:3).

b) Reaction of N-p-tolyl D-isoglucosamine with phenylhydrazine in 2N-acetic acid gave D-glucose phenylosazone, m.p. 208°, in almost quantitative yield.

2.2. L-Glucose Phenylosazone.

The mixture of L-glucose and L-mannose (15g.) obtained by acid decomposition of the product of the condensation of L-arabinose with nitromethane (Sowden & Fischer, 1947), in aqueous solution (250ml.) was warmed on the boiling water bath. Phenylhydrazine (20ml.), glacial acetic acid (14ml.), sodium acetate (4g.), and a few crystals of sodium hydrosulphite (to prevent the formation of dark-coloured contaminants) were added and the mixture heated for 2 hours. The dark brown mixture was cooled and filtered and the residue washed successively with 2N-acetic acid, water, and cold ethanol to yield 6.9g. of dark yellow crystals. Recrystallisation, after treatment with charcoal, from hot absolute ethanol gave pale yellow crystals of L-glucose phenylosazone

(6.0g., 20.0%), m.p. 205-206° (decomp.), mixed m.p. with D-glucose phenylosazone (m.p. 208°) 196°, $[\alpha]_D^{16}$ +57 → +36° (24 hours) [c, 1.00 in pyridine-ethanol (2:3)].

2.3. 3-O-Methyl D-Glucose Phenylosazone.

This osazone was prepared from 3-O-methyl D-glucose according to method a) for D-glucose phenylosazone (2.2.) except that the period of heating was extended to 1 hour. The crude product, m.p. 162-165° (decomp.), was recrystallised from absolute ethanol to give 3-O-methyl D-glucose phenylosazone (77% yield) in the form of pale yellow needles having a double m.p., 167° and 175° (no decomp.). Further recrystallisation from absolute ethanol gave a product m.p. 176°, $[\alpha]_D^{18}$ -105 → -25° (24 hours) [c, 1.00 in pyridine-ethanol (2:3)].

2.4. 3:4:6-Tri-O-Methyl D-Glucose Phenylosazone.

Syrupy 3:4:6-tri-O-methyl D-fructose (16g.) was treated with phenylhydrazine (23ml.) in 2N-acetic acid (300ml.). The mixture was heated on the water bath for 2 hours after which time it was poured into cold water (1 litre). The precipitated oil was dissolved in 99% ethanol and the hot solution treated with charcoal, and filtered. On cooling and dropwise addition of water 3:4:6-tri-O-methyl D-glucose phenylosazone (10.0g., 37%) was obtained as pale yellow crystals, m.p.118° (no decomp.). Recrystallisation from absolute ethanol gave a product showing m.p. 120°, $[\alpha]_D^{18}$ -55 → +31° (75 hours) [c, 1.00 in pyridine-ethanol (2:3)].(Found: C, 62.6; H, 7.1; N, 14.2; OMe, 23.4. $C_{21}H_{28}O_4N_4$ requires C, 63.0; H, 7.0; N, 14.0; 3OMe, 23.3%).

2.5. 3:5:6-Tri-O-Methyl D-Glucose Phenylosazone.

Syrupy 3:5:6-tri-O-methyl D-glucose (32g.) was treated with phenylhydrazine (46ml.) in 2N-acetic acid (450ml.) and the product isolated as described for 3:4:6-tri-O-methyl D-glucose phenylosazone (2.4.). Recrystallisation from absolute ethanol

gave 3:5:6-tri-O-methyl D-glucose phenylosazone as pale yellow crystals (16.0g., 30%), m.p. 62° (no decomp.), $[\alpha]_D^{18}$ -100.0 → -34.5° (70 hours) [c, 1.00 in ethanol-pyridine (2:3)] (Found: C, 62.8; H, 7.2; N, 14.3; OMe, 23.2. $C_{21}H_{28}O_4N_4$ requires C, 63.0; H, 7.0; N, 14.0; 3OMe, 23.3%).

2.6. D-Galactose Phenylosaxone.

This osazone was prepared from D-galactose according to method a) for D-glucose phenylosazone (2. 1.) ; the crude product was recrystallised from absolute ethanol to give D-galactose phenylosazone in 70% yield, m.p. 186° (decomp.)

2.7. L-Gulose Phenylosazone.

L-Sorbose was treated with phenylhydrazine in the manner described for the preparation of the tri-O-methyl hexose phenylosazones (2.2. and 2.3.). Recrystallisation of the crude product from absolute ethanol gave L-gulose phenylosazone in 54% yield, m.p. 164° (decomp.).

2.8. D-Xylose Phenylosazone.

This osazone was prepared from D-xylose according to method a) for D-glucose phenylosazone (2.1.); the crude product was recrystallised from absolute ethanol to give D-xylose phenylosazone, m.p. 166-167° (decomp.), $[\alpha]_D^{17}$ -24.0 → -46.0° (33 hours) [c, 1.00 in pyridine-ethanol (2:3)].

2.9. D-Glucose 2:4-Dinitrophenylosazone.

A solution of D-glucose (6g.) in water (10ml.) was added to a hot solution of 2:4-dinitrophenylhydrazine (20g.) in 2N-hydrochloric acid (1,200ml.) containing ethanol (10ml.) and the mixture heated at 100° for 12 hours, during which time a dark red product was precipitated. The mixture was filtered and the residue washed with cold 2N-hydrochloric acid and water and then dried in a vacuum desiccator to give D-glucose

2:4-dinitrophenylosazone (17.2g., 95%), m.p. 225° (decomp.), after recrystallisation from ethyl acetate m.p. 243° (decomp.).

2.10. D-Glucose Bishydrazone.

N-p-Tolyl-D-isoglucosamine (9g.), hydrazine hydrate (7g.), and 10% acetic acid solution (45ml.) were mixed and warmed on the boiling water bath. A pale yellow oily solution was obtained after 5 minutes heating from which, on further heating, p-toluidine was precipitated as an oil. The mixture was heated for a total of 30 minutes and then cooled whereupon the precipitated p-toluidine crystallised and was removed by filtration. Last traces of p-toluidine were removed by extraction of the solution with ether. In this manner an aqueous solution of D-glucose bishydrazone was obtained.

3. THE PREPARATION OF OSONES.

3.1. By Decomposition of the Corresponding Osazone with Hydrochloric Acid.

3.1.1. D-Glucosone.

D-Glucose phenylosazone was decomposed with concentrated hydrochloric acid strictly according to the directions of Fischer (1889); by this method D-glucosone was obtained in 30% yield.

Attempted decomposition of D-glucose 2:4-dinitrophenylosazone with hydrochloric acid led to the recovery of the starting material.

3.2. By Decomposition of the Corresponding Osazone with Benzaldehyde.

3.2.1. D-Glucosone.

D-Glucose phenylosazone (10g.) was triturated with 96% ethanol (300ml.) and poured into a 2-litre three-necked flask fitted with a dropping funnel, stirrer, and condenser. Glacial acetic acid (6ml.), benzaldehyde (16ml.), and distilled water (500ml.) were added. After refluxing for 2½ hours with constant stirring the solution became clear; refluxing was continued for a further 2 hours, during which benzylidene phenylhydrazone separated out. With the condenser reversed 200ml. of distillate were collected in 30-45 minutes, distilled water (500ml.) being added concurrently through the dropping funnel. The reaction products were syphoned from the flask, allowed to cool overnight and the precipitated benzylidene phenylhydrazone filtered off. The combined filtrate and washings were evaporated under reduced pressure at 40° to a volume of less than 200ml. and extracted 5-6 times with ether (200ml.) to remove residual benzylidene phenylhydrazone, benzoic acid etc. A single treatment with charcoal at this stage gave a faintly greenish-yellow solution, which on evaporation under reduced pressure at 40°

yielded D-glucosone as a pale yellow syrup (2.9g.). The syrup was dissolved in water (8ml.) at 40° and extracted with hot 96% ethanol (300ml.), a further treatment with charcoal carried out and the solution evaporated to a syrup. The syrup was again extracted with ethanol to give a water-white solution. On evaporating to dryness, under reduced pressure, D-glucosone (2.5g., 50.0%) was obtained as a white "froth" which was detached from the flask and dried to constant weight in vacuo over phosphorus pentoxide at 20°; the "froth" showed $[\alpha]_D^{19}$ -10.6 → +4.2° (150 hours) (c, 9.27 in water). (Found: C, 35.8; H, 6.15. $C_6H_{10}O_6$ requires C, 40.4; H, 5.6. $C_6H_{10}O_6.H_2O$ requires C, 36.7; H, 6.1%). Attempts to crystallise the glucosone failed. The osone did not restore the colour of Schiff's reagent and did not decolorise cold, neutral permanganate solution; it reduced Fehling's solution in the cold and gave a blue colour with Benedict's arsenophosphotungstic acid reagent for uric acid in the presence of alkali-cyanide. With phenylhydrazine in acetic acid D-glucose phenylosazone was rapidly formed at room temperature. Further purification of the "froth", achieved by precipitating it from a hot ethanolic solution by addition of ether or by chromatographic purification on a cellulose column using acetone-water (9:1) as developer, brought about no modification in these properties.

Attempted preparation of D-glucosone by decomposition of D-glucose 2:4-dinitrophenylosazone with benzaldehyde according to the above method resulted in quantitative recovery of unchanged osazone.

D-Glucosone was obtained in poor yield by decomposition of D-glucose bishydrazone, prepared in solution, with benzaldehyde; severe losses were incurred during purification.

3.2.2. L-Glucosone.

L-Glucose phenylosazone (6.5g.) was treated with benzaldehyde (10.5ml.) in the manner described for the preparation of D-glucosone.

L-Glucosone, obtained in 45% yield, showed the same chemical properties as D-glucosone, and gave L-glucose phenylosazone, m.p. 204°, on treatment with phenylhydrazine in acetic acid.

3.2.3. 3-O-Methyl D-Glucosone.

A hot solution of 3-O-methyl D-glucose phenylosazone (10g.) in 99% ethanol (150ml.) was added dropwise with stirring during 1 hour to a hot mixture of water (500ml.), glacial acetic acid (6ml.), and benzaldehyde (15ml.). After the addition was complete the reaction mixture was refluxed with constant stirring for 4 hours. Water (150ml.) was then added during the removal of ethanol (135ml.) by distillation. The reaction mixture was syphoned off and treated as for the preparation of D-glucosone (3.2.1.) to yield syrupy 3-O-methyl D-glucosone (1.9g., 37%), which showed the same chemical properties as D-glucosone; 3-O-methyl D-glucose phenylosazone was obtained, m.p. 174°, on treatment with phenylhydrazine in acetic acid at room temperature.

3.2.4. 3:4:6-Tri-O-Methyl D-Glucosone.

The preparation followed the modification of the general method adopted for the preparation of 3-O-methyl D-glucosone (3.2.3.). 3:4:6-Tri-O-Methyl D-glucosone was obtained from the corresponding phenylosazone in 30% yield as a syrup showing the same chemical properties as D-glucosone; treatment with phenylhydrazine in acetic acid gave 3:4:6-tri-O-methyl D-glucose phenylosazone, m.p. 118°.

3.2.5. 3:5:6-Tri-O-Methyl D-Glucosone.

The preparation was carried out by the method described for the 3:4:6-tri-O-methyl osone, syrupy 3:5:6-tri-O-methyl D-glucosone being obtained in 32% yield showing the same chemical properties as D-glucosone; treatment with phenylhydrazine in acetic acid gave 3:5:6-tri-O-methyl D-glucose phenylosazone, m.p. 59°.

3.2.6. D-Galactosone.

The preparation followed the general method described for D-glucosone (3.2.1.). D-Galactosone was obtained as a "froth"(40%) showing the same chemical properties as D-glucosone; treatment with phenylhydrazine in acetic acid gave D-galactose phenylosazone, m.p. 185°.

3.2.7. L-Gulosone (L-Sorbosone).

The preparation followed the general method described for D-glucosone (3.2.1.). L-Gulosone was obtained as a "froth" in 42% yield, showing the same chemical properties as D-glucosone; treatment with phenylhydrazine in acetic acid gave L-gulose phenylosazone, m.p. 163°.

3.2.8. D-Xylosone.

The preparation followed the modification of the general method adopted for the preparation of 3-O-methyl D-glucosone (3.2.3.). D-Xylosone was obtained as a "froth" in 40% yield, showing the same chemical properties as D-glucosone; treatment with phenylhydrazine in acetic acid gave D-xylose phenylosazone, m.p. 166°.

3.3. By Decomposition of the Corresponding Osazone with Pyruvic Acid.

3.3.1. D-Glucosone.

D-Glucose phenylosazone (10g.) was suspended in 20% ethanol (120ml.) containing pyruvic acid (5g.). The mixture was refluxed with constant stirring for 1 hour. After cooling the precipitated pyruvic acid phenylhydrazone was removed by filtration and the filtrate was then purified and worked up in the manner described for the filtrate obtained at a similar stage in the decomposition of D-glucose phenylosazone with benzaldehyde (3.2.1.). D-Glucosone (3.1g., 62%) was thus obtained showing properties identical with those of samples of the osone prepared by other methods.

3.3.2. D-Galactosone.

By the method described for D-glucosone (3.3.1.) D-galactosone was obtained in 61% yield by decomposition of D-galactose phenylosazone with pyruvic acid.

3.3.3. L-Gulosone.

By the method described for D-glucosone (3.3.1.) L-gulosone was obtained in 58% yield by decomposition of L-gulose phenylosazone with pyruvic acid.

3.3.4. D-Xylosone.

By the method described for D-glucosone (3.3.1.) D-xylosone was obtained in 63% yield by decomposition of D-xylose phenylosazone with pyruvic acid.

3.4. By Decomposition of the Corresponding Osazone with Glyoxal.

3.4.1. D-Glucosone.

D-Glucose phenylosazone (10g.) was suspended in 1% acetic acid (100ml.) containing slightly less than the theoretical amount required of glyoxal (1.65g.). The mixture was heated on the boiling water-bath with constant stirring for 2 hours. After cooling the precipitated mixture of glyoxal hydrazone and glyoxal bishydrazone was removed by filtration; the filtrate was rigorously extracted with ether, treated with charcoal, and evaporated under reduced pressure at 40° to give a syrup of crude D-glucosone (3.5g., 67%). By reaction with methylphenylhydrazine the syrup was shown to contain considerable amounts of unchanged glyoxal, characterised as the bismethylphenylhydrazone of glyoxal which was separated from the methylphenylhydrazone of D-glucosone by fractional crystallisation from aqueous ethanol; the presence of free glyoxal in the product would account for the observation of the regeneration of the colour of Schiff's reagent by the "glucosone". Attempts to modify the procedure whereby decomposition was made more complete met with no success; pure glucosone was obtained by

this method only after chromatographic purification of the product on a cellulose column, or by condensation of the product with acetone followed by hydrolysis of the resulting crystalline tri-O-isopropylidene derivative of the osone.

3.5. D-Glucosone by Direct Oxidation of D-Fructose.

3.5.1. Action of Cupric Acetate.

Fructose (18g.) was dissolved in a saturated solution (500ml.) of cupric acetate in water. Solid cupric acetate (100g.) was added and the mixture maintained at 50°, with occasional shaking, for 30 hours, a further 50g. of acetate being added after 20 hours and another 50g. after 25 hours. The reaction mixture was cooled and filtered and the filtrate and washings treated with hydrogen sulphide, sodium chloride (1g.) being added to prevent the formation of colloidal sulphur. The precipitated copper sulphide was filtered and the filtrate and washings aerarted to remove excess hydrogen sulphide. The resultant acid solution was deionised by passage through columns of ion-exchange resins (Amberlite I.R. 100-H and Amberlite I.R. 4B-OH) The effluent was shown to be free of copper ions and of hydrogen sulphide but still reacted slightly acid owing to the presence of acetic acid which is not removed by the weak base exchanger Amberlite I.R. 4B-OH; the use of a strong base exchanger such as Amberlite I.R. 400-OH is precluded since it was shown that strong retention of the osone, as well as reducing sugars in general, occurs on this resin. Chemical reactions indicated the presence of considerable amounts of unoxidised fructose as well as glucosone in the solution. The solution was evaporated under reduced pressure at 40° to a syrup (11.1g.) which was treated with dry acetone (220ml.) containing concentrated sulphuric acid (7ml.) for 18 hours at room temperature. The resulting solution was decanted from traces of undissolved syrup and neutralised, with cooling, with solid anhydrous sodium carbonate; the undissolved syrup was further treated with acetone

containing sulphuric acid, the resulting solution neutralised and combined with that obtained initially. After filtration the combined solutions were evaporated under reduced pressure to a semi-crystalline syrup (14.0g.). The syrup was dissolved in chloroform (150ml.) and the solution rigorously extracted with water. A small portion of the aqueous extract was treated with 1 drop of concentrated hydrochloric acid and boiled; after neutralisation the hydrolysate gave no colour with the arseno-phosphotungstic acid reagent in the presence of alkali-cyanide, thus indicating the absence of glucosone, and gave a positive result on application of Seliwanoff's test, indicating the presence of fructose. The extracted chloroform solution was evaporated to a semi-crystalline syrup (11.1g.) which was shown to contain considerable amounts of isopropylidene derivatives of fructose. The syrup was therefore extracted with hot water and the aqueous extract was combined with that of the chloroform solution; on concentration of the combined aqueous extracts 2:3-4:5-di-O-isopropylidene D-fructose (8.5g.) separated, m.p. 96° after recrystallisation from aqueous ethanol. Recrystallisation of the syrupy residue of the second aqueous extraction from methanol gave tri-O-isopropylidene D-glucosone hydrate (0.35g.), m.p. 124-125°, mixed m.p. with an authentic specimen of the isopropylidene derivative (m.p. 125°) 125°. Since the tri-O-isopropylidene derivative is given by pure D-glucosone in yields of 12-15% the production of 0.35g. of the derivative represents the presence of approximately 1.5g. of free glucosone among the products of oxidation of 18g. of fructose, thus indicating the unsuitability of this particular procedure for the preparation of osones.

Fructose (9g.) was dissolved in methanol (300ml.) containing cupric acetate (4g.) in suspension; the mixture was refluxed for 10 minutes , filtered and the filtrate freed of excess of copper reagent by treatment with hydrogen sulphide. After filtration from the precipitated sulphide the filtrate was freed

of excess of hydrogen sulphide as in the procedure described above. The solution was evaporated under reduced pressure to a syrup (8.8g.) which was treated with acetone (300ml.) containing concentrated sulphuric acid (5ml.) and the product (9.0g.) isolated as described above. The semi-crystalline product was extracted with hot water and the residue was recrystallised from methanol to give tri-O-isopropylidene D-glucosone hydrate (0.51g.), m.p. 124°, thus indicating the formation of approximately 3.0g. of D-glucosone by the oxidation of 9.0g. of D-fructose by this method. Similar oxidation of 90g. of fructose gave 5.0g. of the crystalline tri-O-isopropylidene derivative of D-glucosone. The method has also been applied to the preparation of L-gulosone, 5.1g. of tri-O-isopropylidene L-gulosone hydrate being obtained from the products of the oxidation of 90g. of L-sorbose.

3.5.2. Action of Selenious Acid.

D-Fructose was oxidised with selenious acid according to the directions of Dixon & Harrison (1932) to give D-glucosone in 8.0% yield; osone prepared in this manner showed the same chemical properties as did the sample obtained by the benzaldehyde method (3.2.1.).

3.6. Indirect Syntheses of D-Glucosone.

3.6.1. Oxidation of 2:3:4:6-Tetra-O-Acetyl-2-Oxy-D-Glucal.

a) Tetra-O-acetyl-2-oxy-D-glucal (15.0g.) was added to an ethereal solution (120ml.) of perbenzoic acid (10g.), prepared by the method of Tiffeneau (1928), previously cooled in ice; the mixture was shaken until solution was complete. After 24 hours there separated a crystalline substance (8.0g.), m.p. 62°, identified as unchanged starting material; on concentration of the filtrate 2:3:4:6-tetra-O-acetyl D-glucosone hydrate (5.2g.) separated, after recrystallisation from chloroform-light petroleum m.p. 118° $[\alpha]_D^{18}$ +14.7 → +53.9°(96 hours) (c, 2.00 in 20% ethanol) [Found: O-Ac (by direct titration), 59.9. Calc. for 4 O-Ac, 47.3%].

b) Tetra-O-acetyl-2-oxy-D-glucal (5.0g.) in dry ether (100ml.) was treated at 0° with dry chlorine until the solution became permanently yellow; the solution was evaporated under reduced pressure to a syrup which was dissolved in ether (50ml.) and the solution treated with silver carbonate (10g.) and a few drops of water. The mixture was shaken for 3 hours, filtered and the residue extracted with hot chloroform. The chloroform extract was evaporated under reduced pressure to a syrup which on recrystallisation from chloroform-light petroleum gave 2:3:4:6-tetra-O-acetyl D-glucosone hydrate (1.1g., 21.0%), m.p. 117°, mixed m.p. with product of preparation a) (m.p. 118°) 117°.

c) Tetra-O-acetyl-2-oxy-D-glucal (5.0g.) was chlorinated as described in preparation b); the syrupy product was dissolved in ether (50ml.) and to the solution 1 "teaspoonful" of sodium bicarbonate and a few drops of water were added. The mixture was shaken until evolution of carbon dioxide ceased (½ hour) and then filtered; by extraction of the residue with chloroform a small amount of tetra-O-acetyl D-glucosone hydrate was obtained by evaporation of the extract and recrystallisation of the residue from chloroform-light petroleum. The ethereal filtrate was concentrated and allowed to stand at room temperature for 12 hours; the crystalline precipitate was removed by filtration, further amounts being obtained by addition of light petroleum to the mother-liquor. The crystals were recrystallised from chloroform-light petroleum to give 3:4:6-tri-O-acetyl D-glucosone hydrate (2.3g., 47%), m.p. 75°, $[\alpha]_D^{18}$ +101.4 → +86.3° (10 days) (c, 1.00 in 40% ethanol).

Both tetra-O-acetyl and tri-O-acetyl D-glucosone hydrate were shown to reduce Fehling's solution at room temperature; the tetra-O-acetyl derivative gave a blue colour with Benedict's arsenophosphotungstic acid reagent in the presence of alkali-cyanide on standing, while the tri-O-acetyl derivative gave the colour immediately. After short treatment with dilute alkali both derivatives gave D-glucose phenylosazone, m.p. 204°, on

treatment with phenylhydrazine in acetic acid. A small portion of the tetra-O-acetyl derivative was catalytically deacetylated with sodium in absolute methanol; D-glucosone was detected in the resultant solution by chromatography on paper using the upper phase of a n-butanol - acetic acid - water (4:1:5) mixture as developer and triphenyltetrazolium chloride as identification reagent.

Treatment of tri-O-acetyl D-glucosone hydrate (1.0g.) with acetone (50ml.) containing concentrated sulphuric acid (1.6ml.) gave tri-O-isopropylidene D-glucosone, hydrate, m.p. 125°, isolated in the usual manner (0.1g., 10%).

3.6.2. Oxidation of 2:3-4:5-Di-O-isoPropylidene D-Fructose.
a) From the treatment of the fructose derivative in methanol with cupric acetate at 100° for 10 minutes the starting material was recovered quantitatively.

b) A solution of di-O-isopropylidene fructose (2.0g.) in dry chloroform (50ml.) was treated with manganese dioxide (10g.) and the mixture stored at room temperature for 6 days. The mixture was filtered from the oxidant and the filtrate evaporated under reduced pressure to a semi-crystalline syrup; recrystallisation from 25% ethanol gave di-O-isopropylidene fructose (1.8g.), m.p. 96°. The mother liquor did not regenerate the colour of Schiff's reagent, reduce Benedict's reagent, or form a crystalline derivative on addition of a solution of dimedone.

c) Treatment of a solution of di-O-isopropylidene fructose in light petroleum containing solid calcium carbonate with a solution of tert.-butyl chromate, prepared according to the directions of Oppenauer & Oberrauch (1949), gave a solution showing reducing properties; no crystalline derivative was isolated.

3.6.3. Oxidation of N-p-Tolyl-D-isoGlucosamine.
N-p-Tolyl-D-isoglucosamine (1.0g.) was suspended in water (50ml.) containing bromine (1.0ml.) and the mixture shaken at room

temperature for 1 hour. The clear yellow solution was decanted from the brown tarry material (identified after purification by distillation in steam as 2:6-dibromo-p-toluidine) and freed of excess of bromine by treatment with a stream of nitrogen. The colourless solution was made alkaline to Congo red by addition of sodium hydroxide solution; a small portion of the neutralised solution gave D-glucose phenylosazone, m.p. 205° after recrystallisation from ethanol, on treatment with phenylhydrazine at room temperature. The neutralised solution reduced Fehling's solution without the application of heat, and gave a negative result to Seliwanoff's test; with the arsenophophotungstic acid reagent in the presence of alkali-cyanide a blue colour was given immediately. Chromatographic analysis on paper of the solution showed the presence of glucosone. The solution was evaporated under reduced pressure to a semi-crystalline syrup which was extracted with hot absolute ethanol. The ethanolic extract was evaporated under reduced pressure and the syrupy residue treated with acetone (50ml.) containing concentrated sulphuric acid (1.6ml.). A syrupy product was isolated in the usual manner which on recrystallisation from methanol gave tri-O-isopropylidene D-glucosone hydrate, m.p. 123°, mixed m.p. with authentic specimen of the derivative (m.p. 125°) 124°, in very low yield.

4. NITROGENOUS DERIVATIVES OF GLUCOSONE.

4.1. Attempted Formation of a Semi-Carbazone of D-Glucosone.

Semi-carbazide hydrochloride (0.5g.) was dissolved in water (0.5ml.) and made up to 25ml. with methanol. This solution (20ml.) was neutralised with sodium methylate solution (3.2ml.) prepared by dissolving sodium (0.5g.) in absolute methanol (20ml.). The neutralised solution (4ml.) was added to D-glucosone (0.1g.) and the mixture refluxed for 1 hour. No crystalline product was obtained.

4.2. Attempted Formation of an Oxime of D-Glucosone.

Hydroxylamine hydrochloride (0.4g.) was dissolved in the minimum of warm water; to the solution was added sodium (0.15g.) in absolute methanol (2ml.). The solution was allowed to cool and decanted from the precipitated sodium chloride. To the combined filtrate and washings (2ml. methanol) D-glucosone (0.8g.) was added and the mixture warmed at 30-40°. No crystalline product was obtained.

4.3. D-Glucosone Methylphenylhydrazone.

A solution of D-glucosone (5.0g.) in hot 96% ethanol (35ml.) was treated with methylphenylhydrazine (2.25ml., 3.4g.); the mixture was allowed to stand at room temperature for 2 hours during which time precipitation of yellow crystals occurred. The reaction mixture was stored at 0° over-night, filtered and the residue recrystallised from absolute ethanol to give D-glucosone methylphenylhydrazone (3.1g., 40.0%), m.p. 170°, $[\alpha]_D^{18}$ -260° (c, 1.00 in pyridine)(Found: C, 55.4; H, 6.4; N, 10.0. $C_{13}H_{18}O_5N_2$ requires C, 55.3; H, 6.4; N, 9.9%). The derivative gave no colour with the arsenophosphotungstic acid reagent in the presence of alkali-cyanide, did not regenerate the colour of Schiff's reagent, and gave a negative result on application of Seliwanoff's test; it reduced Fehling's

solution only on boiling. On refluxing a solution of the derivative with benzaldehyde or on dissolving it in cold concentrated hydrochloric acid solutions were obtained giving qualitative tests for glucosone.

4.4. Reaction of D-Glucosone with 2:5-Dichlorophenylhydrazine.

D-Glucosone (1.0g.) was added to a solution of 2:5-dichlorophenylhydrazine (1.0g.) in hot methanol (8ml.) to give an immediate precipitate of bright yellow crystals. The product was removed by filtration, washed once with ether and once with water; recrystallisation from aqueous ethanol gave D-glucose 2:5-dichlorophenylosazone (1.0g., 70.0% calculated on 2:5-dichlorophenylhydrazine), m.p. 232°, mixed m.p. with authentic specimen (m.p. 233°) 232° (Found: C, 44.4; H, 3.7; N, 10.1. $C_{18}H_{18}O_4N_4Cl_4$ requires C, 44.5; H, 3.7; N, 9.9%). Under the conditions described above, even in the presence of a large excess of the phenylhydrazine derivative, glucose and fructose were shown to give the corresponding 2:5-dichlorophenylhydrazones, m.p. 160° and 151° respectively; addition of the reagent to a solution containing both glucosone and glucose gave an immediate precipitate of the osazone, derived from the osone, the hydrazone of glucose being obtained only on concentration of the reaction mixture.

5. METHODS OF ESTIMATION OF GLUCOSONE.

5.1. Gravimetric Estimation as Glucose 2:4-Dinitrophenylosazone.

Tri-O-isopropylidene D-glucosone hydrate (0.1500g.) was hydrolysed with 0.1N-sulphuric acid at 100°. When solution was complete the hydrolysate was made up to a volume of 25ml. with water; 1ml. of the standard solution was diluted with water and made up to a volume of 100ml. 25ml. of the dilute solution was treated with 10ml. of a 1.5% solution of freshly recrystallised 2:4-dinitrophenylhydrazine in 2N-hydrochloric acid at 40° for 3 hours. The precipitated D-glucose 2:4-dinitrophenylosazone was filtered off on a tared sintered-glass funnel, washed with a little 2N-hydrochloric acid and then with cold water and dried to constant weight in a vacuum desiccator. Yield : 24.5mg.; theoretical yield : 25.5mg., i.e. 96% yield obtained.

Similar treatment of a standard aqueous solution of D-glucosone "froth", calculated as glucosone monohydrate $C_6H_{10}O_6.H_2O$, gave 81% of the theoretical yield of D-glucose 2:4-dinitrophenylosazone.

5.2. Estimation of the Reducing Power of Glucosone by Nelson's Method.

Comparison of the reducing powers of standard solutions of D-glucosone "froth", a hydrolysate of tri-O-isopropylidene D-glucosone hydrate, and D-glucose was made by the method of Nelson (1944), using the improved copper reagent of Somogyi (1945); results are recorded in Fig. 4., p. 136. Glucosone was shown to possess approximately 40% of the reducing power of glucose; the reducing power of the osone "froth" is identical with that of the product of hydrolysis of tri-O-isopropylidene D-glucosone hydrate, the product being calculated as glucosone monohydrate, $C_6H_{10}O_6.H_2O$.

5.3. Estimation of Glucosone Using Benedict's Reagent for Uric Acid.

Standard solutions of D-glucosone "froth" and a hydrolysate of tri-O-isopropylidene D-glucosone hydrate, containing in the order of 0.3mg. of glucosone/ml., were compared colorimetrically with a standard solution of uric acid, employing Benedict's arsenophosphotungstic acid reagent in the presence of alkali--cyanide. 0.5, 1.0, 1.5 and 2.0ml. samples of the standard solutions were mixed with 1.0ml. of the reagent and 1.0ml. of a 5% solution of potassium cyanide in 0.01N-potassium hydroxide containing 10% (w/v) of urea; the mixtures were made up to a volume of 5ml. with water and placed in a boiling water bath for 1 minute; they were then cooled for 1 minute in cold water, allowed to stand at room temperature for 2 minutes, and then diluted to 25ml. and compared in a photoelectric colorimeter (Eel), using an Ilford 621 filter, against a "blank". Results are recorded graphically in Fig. 5., p. 138. The "froth" was shown to possess 83% of the reducing power of the product of the hydrolysis, calculated on the latter being D-glucosone monohydrate, $C_6H_{10}O_6.H_2O$.

Fructose was shown not to interfere with the estimation in concentrations of up to 10mg./ml. ; under the conditions of the estimation solutions of glucose were shown to give no colour with the reagents.

6. PREPARATION AND PROPERTIES OF isoPROPYLIDENE DERIVATIVES OF OSONES.

6.1. D-Glucosone.

6.1.1. Tri-O-isoPropylidene D-Glucosone Hydrate.

D-Glucosone (4.12g.) was dissolved in methanol (50ml.) and the solution evaporated rapidly under reduced pressure at 50° to yield a stiff "froth". Acetone (150ml.), dried over anhydrous calcium chloride and redistilled, was cooled to 0° and concentrated sulphuric acid (5ml.) was added dropwise with stirring. The acidified acetone was added to the osone "froth" and the mixture shaken for 8 hours, after which time solution was complete. The brown solution was allowed to stand overnight in the refrigerator and then neutralised, with cooling, with anhydrous sodium carbonate, filtered and the filtrate evaporated to dryness at atmospheric pressure and finally under reduced pressure at 60°. The residual semi-crystalline syrup was dissolved in absolute methanol (15ml.) and the solution cooled to yield 0.85g. of crystalline material, m.p. 125°. Further crops were obtained by concentration of the mother liquor and by the addition of water to give a total yield of 1.12g. (15.5%). A further small amount of the same material was obtained by chromatographic analysis of the non-crystalline residue following the above procedure. Recrystallisation from methanol or dioxan gave tri-O-isopropylidene D-glucosone hydrate (hereafter referred to as (I)), m.p. 125°, $[\alpha]_D^{15}$ -6.6° (c, 2.12 in methanol) (Found: C, 57.0; H, 7.5; CMe_2, 39.5. $C_{15}H_{24}O_7$ requires C, 56.9; H, 7.6; $3CMe_2$, 39.9%). The crystals were soluble in most organic solvents except light petroleum and insoluble in water.

6.1.2. Attempted Acetylation of Tri-O-isoPropylidene D-Glucosone Hydrate.

a) The compound (I) (0.5g.) was dissolved in redistilled pyridine (3ml.), the solution cooled to 0° and acetic anhydride (3ml.)

added. The mixture was warmed to 30° and allowed to stand overnight at this temperature and then poured, with stirring, into ice water (30ml.). The crystalline precipitate was filtered off, washed with water and recrystallised from methanol yielding colourless crystals (0.45g.), m.p. 125°, not depressed on admixture with authentic crystals of(I).

b)The derivative(I) (0.5g.) was dissolved in acetic anhydride (5ml.), anhydrous sodium acetate (0.1g.) added, and the mixture warmed for 5 hours at 75°. The cooled reaction mixture was poured into ice water (25ml.) and adjusted, with stirring, to pH 6 with sodium bicarbonate. The dried chloroform extract was evaporated to a syrup under reduced pressure and acetic acid was removed by codistillation with toluene. The syrup crystallised from ether-light petroleum (b.p. 40-60°), m.p. 123-124°, not depressed on admixture with authentic specimen of (I).

6.1.3. Attempted Methylation of Tri-O-*iso*Propylidene D-Glucosone Hydrate.

a) The compound (I) (0.75g.) was subjected to four methylations with freshly prepared silver oxide (1.0g.) and dry methyl iodide (3ml.) in the usual way (Purdie & Irvine, 1903). The residual semi-crystalline syrup crystallised from methanol (0.58g.), m.p. 125° not depressed on admixture with authentic (I).

b) The derivative (I) (0.5g.) was dissolved in sodium-dried ether (10ml.). Sodium wire (0.5g.) was added and the mixture refluxed for 6 hours. No evolution of hydrogen was apparent. The solution was decanted from the sodium and, with shaking, dimethyl sulphate (0.3ml.) was added. The mixture was refluxed for 15 minutes and allowed to stand overnight at room temperature. A very slight precipitate resulted. The mixture was worked up in the usual way, the compound (I) (0.4g.) (m.p. and mixed m.p. 124°) being recovered.

6.1.4. Hydrolysis of Tri-O-isoPropylidene D-Glucosone Hydrate

a) The compound (I) (0.2g.) was refluxed with glacial acetic acid (5ml.), water (5ml.) and 0.1N-hydrochloric acid (0.1ml.) for 2 hours when the solution reduced Fehling's solution in the cold and gave a strong blue colour with the arsenophosphotungstic acid reagent in the presence of alkali-cyanide. After neutralisation the solution was evaporated to a brown syrup which with phenylhydrazine in acetic acid gave an immediate precipitate of D-glucose phenylosazone, m.p. 204° after recrystallisation from ethanol.

b) The derivative (I) (0.48g.) was dissolved in 85% acetic acid (10ml.), the solution maintained at 50° and the hydrolysis followed polarimetrically - see Fig. 6., p. 147. After 3 hours a slight coloration with the arsenophosphotungstic acid reagent and a slight reduction of Fehling's solution was observed. After 5 hours the rotation remained constant during a period of 8 hours after which time it continued to rise reaching final equilibrium after a total of 56 hours. After 20 hours a strong colour was obtained with the arsenophosphotungstic acid reagent and the solution strongly reduced Fehling's solution.

c) see 5.1.

6.1.5. Partial Hydrolysis of Tri-OisoPropylidene D-Glucosone Hydrate.

The compound (I) (1.32g.) was dissolved in 85% acetic acid (26ml.) and the solution maintained at 50° for 10 hours. The solution was evaporated at 40° to a pale yellow syrup (1.1g.) and residual acetic acid was removed by co-distillation under reduced pressure with toluene. The syrup was dissolved in a few ml. of hot methanol and water added to turbidity; a small crystalline fraction (0.1g.), identified as unchanged (I), separated out and was removed by filtration. The syrup, which gave only a very faint colour with the arsenophosphotungstic acid reagent and did not reduce Benedict's solution, was

extracted with acetone. The extract was evaporated to a faintly coloured syrup, soluble in most organic solvents except light petroleum and insoluble in cold water. Attempts to crystallise it from various solvents met with no success.

6.1.6. Di-O-Acetyl-Di-O-*iso*Propylidene D-Glucosone Hydrate. The syrup (0.39g.) obtained by partial hydrolysis of the compound (I) was dissolved in acetic anhydride (4.0ml.) and warmed at 75° for 5 hours with anhydrous sodium acetate (0.1g.). The cooled reaction mixture was worked up as in 6.1.2. b), and the resultant syrup (0.47g.) crystallised from ether-light petroleum, colourless aggretated prisms, (0.38g., 75.0%), m.p. 69°. Recrystallisation from aqueous methanol gave di-O-acetyl-di-O--*iso*propylidene D-glucosone hydrate, m.p. 70°, $[\alpha]_D^{19}$ +15.9° (c, 1.44 in methanol) [Found: C, 53.2; H, 6.65; CMe_2, 23.4; OAc, 24.8 (by direct titration). $C_{16}H_{24}O_9$ requires C, 53.3; H, 6.7; $2CMe_2$, 23.2; 2OAc, 23.9%].

6.1.7. Oxidation of Di-O-*iso*Propylidene D-Glucosone Hydrate by Periodate at Room Temperature.

a) Reduction of periodate. Di-O-acetyl-di-O-*iso*propylidene D-glucosone hydrate (0.134g.) was dissolved in 0.1N-sodium hydroxide (12ml.) at 100°. The solution was cooled and neutralised (phenolphthalein) by addition of 0.1N-hydrochloric acid. 0.265M-Sodium periodate (2ml.) was added and the volume adjusted to 20ml. The periodate was determined on samples by the usual iodine-arsenite method; 0.92 mole of periodate was reduced per mole of sugar in 2 hours, and 0.98 mole in 6 hours.

b) Formic acid production. Titration of a 6-hour sample (10ml.) showed that no acid had been formed.

c) Formaldehyde production. The technique employed was that described by Bell (1948). When the solution of di-O-*iso*propylidene D-glucosone hydrate obtained as in a) was oxidised under these conditions the product formed (32.2mg. in 2 hours, 34.8mg. in 24 hours; from 23.2mg. of the di-O-acetyl compound) on

addition of dimedone had m.p. 140-150°. After a single recrystallisation from ethanol (1ml.) the formaldehyde derivative, m.p. 184-185° alone and mixed with an authentic sample, was obtained. 0.79 Mole of formaldehyde was formed per mole of the sugar in 2 hours, and 0.80 mole in 24 hours.

By addition of water to the ethanolic mother liquor a crystalline product, m.p. 158-159° after two recrystallisations from 50% ethanol, was isolated. The expected carbohydrate product is 1:2-2:3-di-O-isopropylidene 5-aldo-D-xylosone hydrate; it would appear that its dimedone derivative is insoluble in water. The carbohydrate nature of the crystalline derivative was demonstrated by a positive result in Molisch's test, and, after hydrolysis with mineral acid, positive results in Benedict's copper reduction test and in the arsenophosphotungstic acid test; acetone was found to be present in the hydrolysate.

6.1.8. Methylation of Di-O-isoPropylidene D-Glucosone Hydrate.
a) Deacetylation. The diacetate (1.0g.) was dissolved in dry methanol (40ml.) and sodium (30mg.) in methanol (6ml.) added. The mixture was allowed to stand at room temperature for 12 hours, and then evaporated under reduced pressure to a volume of 10ml.

b) Methylation. To the above solution dry methyl iodide (10ml.) was added and the mixture refluxed for 1 hour during which time sodium iodide separated out. Anhydrous calcium sulphate (1.0g.) and fresh silver oxide (5.0g.) were added and reflux continued for a further 4 hours. The mixture was extracted with dry acetone (150ml.) and the extract evaporated to a syrup which was subjected to three further methylations. The product was distilled in a vacuum (90-110°/0.05mm.) to yield syrupy di-O-methyl di-O-isopropylidene D-glucosone hydrate (0.37g., 43%), $[\alpha]_D^{20}$ +1.2° (c, 3.3 in methanol) (Found: OMe, 19.5; $C_{14}H_{24}O_7$ requires 20Me, 20.4%).

6.1.9. Hydrolysis of Di-O-Methyl-Di-O-*iso*Propylidene D-Glucosone Hydrate.

A solution of the di-O-methyl derivative (50mg.) in methanol was evaporated to a small volume (0.25ml.), water (1ml.) and concentrated hydrochloric acid (2 drops) added, and the mixture heated at 100° for 10 minutes. The hydrolysate was neutralised by addition of solid sodium acetate. *p*-Bromophenylhydrazine hydrochloride (0.2g.) and sodium acetate (0.2g.) were added and heating continued for a further 10 minutes. On cooling an oil separated out and crystallised on scratching. On recrystallisation from aqueous ethanol the derivative showed m.p. 154°; Salmon & Powell (1939) give m.p. 156° for 5:6-di-O-methyl D-glucose *p*-bromophenylosazone.

6.1.10. Oxidation of 5:6-Di-O-Methyl D-Glucosone.

To N-sulphuric acid (2ml.) in an open dish heated on the boiling water bath was added dropwise a solution of di-O-methyl-di-O--*iso*propylidene D-glucosone hydrate (150mg.) in methanol (4.5ml.). After 15 minutes heating to remove methanol the yellow solution was transferred to a conical flask and cooled. 10% Sodium metaperiodate (6ml.) was added and the mixture set aside at room temperature for 72 hours. From the reaction product, by the method of Salmon & Powell (1939), there was obtained a solution of αβ-dimethoxypropionic acid, identified as its *p*-bromophenacyl derivative (150mg., 64.5%).

(N.B. Alternative methods of preparation of tri-O-*iso*propylidene D-glucosone hydrate are outlined in Part II, 3.2.2.1.).

6.2. L-Glucosone.

6.2.1. Tri-O-*iso*Propylidene L-Glucosone Hydrate.

When L-glucosone (1.0g.) was treated with acetone as described in 6.1.1., crystalline tri-O-*iso*propylidene L-glucosone hydrate (0.2g., 11.5%) was obtained, m.p. 125°, $[\alpha]_D^{16}$ +6.8° (c, 2.00 in methanol) (Found: C, 57.1; H, 7.6%).

6.3. 3-O-Methyl D-Glucosone.

Treatment of 3-O-methyl D-glucosone with acetone as described in 6.1.1. gave a product, isolated in the usual manner, which could not be crystallised. The derivative was non-reducing, was insoluble in water, and gave qualitative tests for an isopropylidene derivative; decomposition occurred on attempted distillation in a vacuum. On hydrolysis with dilute mineral acid 3-O-methyl D-glucosone was obtained, characterised as 3-O-methyl D-glucose phenylosazone, m.p. 174°.

6.4. L-Gulosone.

6.4.1. Tri-O-isoPropylidene L-Gulosone Hydrate.
When L-gulosone (4.0g.) was treated with acetone in the manner described in 6.1.1. gave a crystalline product (0.9g., 13.0%), showing physical and chemical properties similar to those of tri-O-isopropylidene D-glucosone hydrate. Recrystallisation from methanol gave tri-O-isopropylidene L-gulosone hydrate, m.p. 133°, $[\alpha]_D^{18}$ -20.4° (c, 2.25 in methanol) (Found: C, 57.1; H, 7.5; CMe_2, 39.6. $C_{15}H_{24}O_7$ requires C, 56.9; H, 7.6; CMe_2, 39.9%).

6.4.2. Hydrolysis of Tri-O-isoPropylidene L-Gulosone Hydrate.
a) Treatment of the tri-O-isopropylidene derivative in the manner described in 6.1.4. a) gave a syrupy product which with phenylhydrazine in acetic acid gave L-gulose phenylosazone, m.p. 163°.

b) The derivative (0.5g.) was dissolved in 85% acetic acid (10ml.) the solution maintained at 50° and the hydrolysis followed polarimetrically - see Fig. 7., p. 147. After 3 hours the rotation remained constant during a period of 3 hours after which time it decreased reaching final equilibrium after a total of 70 hours. After 15 hours a strong colour was obtained with the arsenophosphotungstic acid reagent and the solution strongly reduced Fehling's solution.

c) After hydrolysis with 0.1N-sulphuric acid a 94% yield of

L-gulose 2:4-dinitrophenylosazone was obtained on treatment with a 1.5% solution of 2:4-dinitrophenylhydrazine in 2N-hydrochloric acid according to the directions described in 5.1.

6.4.3. Partial Hydrolysis of Tri-O-*iso*Propylidene L-Gulosone Hydrate.

The tri-O-*iso*propylidene derivative (1.05g.) was dissolved in 85% acetic acid (20ml.) and the solution maintained at 50° for 4 hours. The solution was evaporated at 40° to a pale yellow syrup (0.8g.) and residual acetic acid was removed by co-distillation under reduced pressure with toluene. The syrup, which gave only a very faint colour with the arsenophosphotungstic acid reagent and did not reduce Fehling's solution, was extracted with acetone. The extract was evaporated to a syrup, soluble in most organic solvents except light petroleum and insoluble in cold water. Attempts to crystallise it from various solvents met with no success.

6.4.4. Di-O-Acetyl-Di-O-*iso*Propylidene L-Gulosone Hydrate.

The syrup (0.75g.) obtained as in 6.4.3. was dissolved in acetic anhydride (3.0ml.) and warmed at 75° for 5 hours with anhydrous sodium acetate (0.2g.). The cooled reaction mixture was worked up as in 6.1.2. b), and the resultant syrup crystallised from aqueous methanol to give di-O-acetyl-di-O-*iso*propylidene L-gulosone (0.70g., 72.0%), m.p. 94°, $[\alpha]_D^{17}$ +22.5° (c, 1.50 in methanol) [Found: C, 53.35; H, 6.65; CMe_2, 23.1; OAc, 24.5 (by direct titration). $C_{16}H_{24}O_9$ requires C, 53.3; H, 6.7; $2CMe_2$, 23.2; 2OAc, 23.9%].

6.4.5. Oxidation of Di-O-*iso*Propylidene L-Gulosone Hydrate by Periodate at Room Temperature.

a) Reduction of periodate. An alkaline hydrolysate of di-O-acetyl-di-O-*iso*propylidene L-gulosone hydrate (0.120g.) was treated with 0.265M-sodium periodate (2ml.) in the manner described for the corresponding derivative of D-glucosone (6.1.7. a). The periodate was determined on samples by the usual iodine-

-arsenite method; 0.90 mole of periodate was reduced per mole of sugar in 2 hours, and 0.96 mole in 6 hours.

b) Formic acid production. Titration of a 6-hour sample (10ml.) showed that no acid had been formed.

c) Formaldehyde production. By the technique described in 6.1.7. c) it was shown that 0.82 (0.91) mole of formaldehyde was formed per mole of the sugar in 24 hours.

From the ethanolic mother liquor of the recrystallisation of the dimedone derivative of the formaldehyde formed during the oxidation was obtained a crystalline product, m.p. 157°, mixed m.p. with corresponding derivative obtained from the oxidation of di-O-isopropylidene D-glucosone hydrate (m.p. 158-159°) 157°.

6.5. D-Xylosone.

Treatment of D-xylosone with acetone as described in 6.1.1. gave a product, isolated in the usual manner, which could not be crystallised. The derivative was non-reducing, was insoluble in water, and gave qualitative tests for an isopropylidene derivative. On hydrolysis with dilute mineral acid D-xylosone was obtained, characterised as D-xylose phenylosazone, m.p. 166°.

SUMMARY.

1. A critical and comprehensive review of the published work on the preparation, properties, structure, and biological significance of the osones is presented.

2. A full experimental investigation of the methods of preparation of osones is reported. By the introduction of modifications established methods have been adapted for the preparation of osones, possessing a high degree of purity, in higher yields than hitherto obtained; a number of new preparative procedures are described.

3. The physical properties of glucosone, including rotational behaviour, ultraviolet absorption spectrum, chromatographic analysis, and ionophoretic analysis of aqueous solutions, properties which have hitherto received little or no attention, have been studied; the results are discussed in relation to the structural features of the osone and to the detection and identification of glucosone when in admixture with other sugars or in biological material.

4. The effect of acids and of alkalis on glucosone has been investigated and the preparation and properties of a number of osone derivatives with substituted hydrazines are described. Methods of estimation of glucosone in the presence of other sugars have been evolved.

5. The preparation, properties, and structures of isopropylidene derivatives of a number of osones are described. 1:2-2:3-5:6-Tri-O-isopropylidene D- and L-glucosone hydrate and 1:2-2:3-5:6-tri-O-isopropylidene L-gulosone hydrate were obtained, the first crystalline derivatives from which the corresponding osones may be readily regenerated.

6. The possible structures which may be assigned to D-glucosone on the available evidence are discussed.

REFERENCES.

Titles of journals are abbreviated in accordance with the system used in the World List of Scientific Periodicals (1952, 3rd. ed., Oxford: University Press).

Anderson, E. (1909). Amer. chem. J. 42, 401.

Anderson, C. G., Charlton, W. & Haworth, W. N. (1929). J. chem. Soc. p. 1329.

Angelli, A. & Marchetti, G. (1908). R.C. Acad. Lincei, (v), 17, 360.

Antoniani, C. (1935). Biochim. Terap. sper. 22, 597.

Ariyama, K. (1927). J. biol. Chem. 74, xiv.

Arnstein, H. R. V. & Bentley, R. (1953). Biochem. J. 54, 493.

Ault, R. G., Baird, D.K., Carrington, H. C., Haworth, W.N., Herbert, R., Hirst, E. L., Percival, E. G. V., Smith, F., & Stacey, M. (1933). J. chem. Soc. p.1419.

Bacon, J. S. D. & Bell, D. J. (1948). Biochem. J. 42, 397.

Baer, E. (1940). J. Amer. chem. Soc. 62, 1597.

Baird, D. K., Haworth, W. N., Herbert, R., Hirst, E. L., Smith, F. & Stacey, M. (1934). J. chem. Soc. p. 62.

Barham, H. N. & Smits, B. L. (1934). Trans. Kans. Acad. Sci. 37, 91.

Barnes, R. P. & Tulane, V. J. (1940). J. Amer. chem. Soc. 62, 894.

Bayne, S. (1952a). Biochem. J. 50, xxvii.

Bayne, S. (1952b). J. chem. Soc. p. 4993.

Bayne, S. (1953). Congress of Carbohydrate Chemistry, London.

Bayne, S., Collie, G. A. & Fewster, J. A. (1952). J. chem. Soc. p. 2766.

Becker, C. E. & Day, H. G. (1953). J. biol. Chem. 201, 795.

Becker, C. E. & May, C.E. (1949). J. Amer. chem. Soc. 71, 1491.

Bednarczyk, W. & Marchlewski, L. (1937). Bull. int. Acad. pol. Sci. A, p. 140.

Bednarczyk, W. & Marchlewski, L. (1938). Bull. int. Acad. pol. Sci. A, p. 524.

Bell, D. J. (1936). J. chem. Soc. p.186.

Bell, D. J. (1947). J. chem. Soc. p. 1461.

Bell, D. J. (1948). J. chem. Soc. p. 992.

Bergmann, M. & Zervas, L. (1931). Ber. dtsch. Ges. 64, 1434, 2032.

Berkeley, C. (1933). Biochem. J. 27, 1357.

Bollinger, H. R. & Prins, D. A. (1945). Helv. chim. acta, 28, 465.

Bond, C. R., Knight, E. C. & Walker, T. K. (1937). Biochem. J. 31, 1033.

Bonner, W. A. (1951). Advanc. Carbohyd. Chem. 6, 251.

Bourne, E. J., McSweeney, G. P. & Wiggins, L. F. (1952). J. chem. Soc. p. 3113.

Bourne, E. J. & Wiggins, L. F. (1948). J. chem. Soc. p. 1933.

Brüll, L. (1936). Ann. Chim. appl. 26, 415.

Brüll, L. (1937). Ric. sci. I, 8, 527.

Busch, M. (1941). Germ. Pat. 702, 729.

Carpéni, G. (1938a). C.R. Acad. Sci., Paris, 206, 1376.

Carpéni, G. (1938b). C.R. Acad. Sci., Paris, 206, 1571.

Case, E. M. & Cook, R. P. (1931). Biochem. J. 25, 1319.

Ciamician, G. & Silber, P. (1901). Ber. dtsch. chem. Ges. 34, 1530.

Ciamician, G. & Silber, P. (1913). Ber. dtsch. chem. Ges. 46, 3894.

Collie, G. A. (1941). Ph. D. thesis, University of St. Andrews.

Consden, R. & Stanier, W. M. (1952). Nature, Lond., 169, 783.

Cook, E. W. (1941). Canad. Pat. 398, 202.

Cramer, M. & Cox, H. (1922). Helv. chim. Acta, 5, 884.

Cross, C. F., Bevan, E. J. & Smith, C. (1898). J. chem. Soc. 73, 463.

Dixon, K. C. & Harrison, K. (1932). Biochem. J. 26, 1954.

Dalmer, O. & Heyns, K. (1939). U.S. Pat. 2, 190, 377.

Dreywood, R. (1946). Ind. Engng Chem. (Anal.) 18, 499.

Durrum, E. L. (1950). J. Amer. chem. Soc. 72, 2943.

Efendi, P. G. & Ryzhova, A. P. (1939). Bull. Biol. Med. exp. URSS, 7, 335.

Ehrlich, F. & Guttmann, R. (1934). Ber. dtsch. chem. Ges. 67, 573.

Engel, L. L. (1935). J. Amer. chem. Soc. 57, 2419.

Eustigneev, V. B. & Nickiforova, V. N. (1950). Biokhim. khain. Proiz. 15. 86.

Evans, W. L. (1929). Chem. Rev. 6, 291.

Evans, W. L. (1942). Chem. Rev. 31, 537.

Evans, W. L., Nicoll, W.D., Strause, G. C. & Waring, C. E. (1928). J. Amer. chem. Soc. 50, 2267.

Evans, W. L. & Waring, C. E. (1926). J. Amer. chem. Soc. 48, 2678.

Fearon, W. R. (1950). Analyst, 75, 56.

Fenton, H. J. H. (1894). J. chem. Soc. 65, 899.

Fenton, H. J. H. (1895). J. chem. Soc. 66, 774.
Fenton, H. J. H. (1896). J. chem. Soc. 69, 546.

Fenton, H. J. H. & Jackson, H. (1899). J. chem. Soc. 75, 1.

Fischer, E. (1887). Ber. dtsch. chem. Ges. 20, 821.

Fischer, E. (1888). Ber. dtsch. chem. Ges. 21, 2631.

Fischer, E. (1889). Ber. dtsch. chem. Ges. 22, 87.

Fischer, E. & Armstrong, E. F. (1902). Ber. dtsch. chem. Ges. 35, 3141.

Fischer, E.& Tafel, J. (1889). Ber. dtsch. chem. Ges. 22, 97.

Fischer, E. & Zemplén, G. (1909). Liebigs Ann. 365, 1.

Fischler, F. (1927). Hoppe-Seyl. Z. 165, 68.

Fleury, P. & Fievet-Guinard, Y. (1947). Ann. pharm. franc. 5, 404.

Foster, A. B. (1952). Chem. & Ind. (Rev.), p. 828.

Foster, A. B. (1953). J. chem. Soc. p. 982.

Foster, A. B. & Stacey, M. (1953). J. appl. Chem. 3, 19.

Freudenberg, K. & Hixon, R. M. (1923). Ber. dtsch. chem. Ges. 56, 2119.

Glattfield, J. W. & Gershon, S. (1938). J. Amer. chem. Soc. 60, 2013.

Glen, W. I., Myers, G. S. & Grant, G. A. (1951). J. chem. Soc. p. 2568.

Goebel, W.F. (1927). J. biol. Chem. 72, 809.

Gottschalk, A. (1952). Biochem. J. 52, 455.

Griess, P. & Harrow, G. (1887). Ber. dtsch. chem. Ges. 20, 2205.

Grunenberg, H. V., Bredt, C. & Freudenberg, W. (1938). J. Amer. chem. Soc. 60, 1507.

Haber, F. & Weiss, J. (1934). Proc. Roy. Soc. A, 147, 333.

Hamilton, J. K. & Smith, F. (1952). J. Amer. chem. Soc. 74, 5162.

Hann, R. M. & Hudson, C. S. (1944). J. Amer. chem. Soc. 66, 1909.

Hartley,F. & Linnell, W. H. (1939). Quart. J. Pharm. 12, 743.

Hartley, F. & Linnell, W. H. (1940). Quart. J. Pharm. 13, 150.

Haskins, W. T., Hann, R. M. & Hudson, C. S. (1942). J. Amer. chem. Soc. 64, 137.

Haskins, W. T., Hann, R. M. & Hudson, C.S. (1946). J. Amer. chem. Soc. 68, 1767.

Haworth, W. N., Hirst, E. L. & Chamberlain, K. A. (1937). J. chem. Soc. p. 795.

Haworth, W. N., Hirst, E. L. & Jones, J. K. N. (1937). J. chem. Soc. p. 552.

Haworth, W. N., Hirst, E. L. & Jones, J. K. N. (1938). J. chem. Soc. p. 710.

Haworth, W. N., Hirst, E. L., Jones, J. K. N. & Smith, F. (1934). J. chem. Soc. p. 1192.

Haworth, W. N., Hirst, E. L. & Percival, E. G. V. (1932). J. chem. Soc.p. 2384.

Haworth, W. N. & Jones, J. K. N. (1944). J. chem. Soc. p. 667.

Haworth, W. N. & Streight, R. L. (1932). Helv. chim. acta, 15, 609.

Heddle, W. J. & Percival, E. G. V. (1939). J. chem. Soc. p. 249.

Helferich, B. & Himmen, E. (1928). Ber. dtsch. chem. Ges. 61, 1825.

Henze, M. (1931a). Z. phys. Chem. 198, 82.

Henze, M. (1931b). Z. phys. Chem. 200, 232.

Herbert, R., Hirst, E. L., Percival, E. G. V., Reynolds, R.W. & Smith, F. (1933). J. chem. Soc. p. 1270.

Herring, P. T. & Hynd, A. (1928). J. Physiol. 66, 267.

Herzfeld, P. (1895). Ber. dtsch. chem. Ges. 28, 440.

Heyns, K. (1947). Liebigs Ann. 558, 171.

Heyns, K. & Heinemann, R. (1947). Liebigs Ann. 558, 187.

Hönig, N. & Tempus, F. (1924). Ber. dtsch. chem. Ges. 57, 787.

Hough, L., Jones, J. K. N. & Wadmann, W. H. (1949). J. chem. Soc. p. 2511.

Hudson, C. S. (1903). Z. phys. Chem. 44, 487.

Hudson, C. S. (1945). Advanc. Carbohyd. Chem. 1, 1.

Hudson, C. S. (1951). Proc. Amer. chem. Soc. 10Q.

Hurd, C. D. & Isenhour, L. L. (1932). J. Amer. chem. Soc. 54, 322.

Hynd, A. (1927a). Proc Roy. Soc. B, 101, 244.

Hynd, A. (1927b). Biochem. J. 21, 1094.

Hynd, A. (1930). Proc. Physiol. Soc., J. Physiol. 70.

Hynd, A. (1931). Biochem. J. 25, 11.

Irvine, J. C. & Garrett, C. S. (1910). J. chem. Soc. 98, 1283.

Irvine, J. C. & Macdonald, J. (1915). J. chem. Soc. 107, 1710.

Irvine, J. C. & Patterson, B. M. (1922). J. chem. Soc. 121, 2146.

Irvine, J. C. & Scott, J. P. (1913). J. chem. Soc. 103, 564.

Isbell, H. S. (1942). J. Res. nat. Bur. Stand. 29, 227.

Isbell, H. S. (1943). Annu. Rev. Biochem. 12, 205.

Isbell, H. S. (1944). J. Res. nat. Bur. Stand. 32, 45.

Isbell, H. S. & Frush, H.L. (1931). J. Res. nat. Bur. Stand. 6, 1145.

Isbell, H. S. & Pigman, W. W. (1937). J. Res. nat. Bur. Stand. 18, 141.

Isbell, H. S. & Pigman, W. W. (1938). J. Res. nat. Bur. Stand. 20, 773.

Isherwood, F. A. (1953). Congress of Carbohydrate Chemistry, London.

Iwadare, M. (1941). Bull. chem. Soc. Japan, 16, 40.

Jaenicke, W, (1952). Naturwissenschaften, 39, 86.

Johnstone, J. H. & Mitchell, I. L. S. (1953). Biochem. J. 55, xvii.

Jolles, A. (1911). Biochem. Z. 34, 242.

Kermack, W. O., Lambie, C. G. & Slater, R. H. (1929). Biochem. J. 23, 410.

Kiliani, H. (1882). Ber. dtsch. chem. Ges. 15, 701.

Kiliani, H. (1885). Ber. dtsch. chem. Ges. 18, 631.

Kiliani, H. & Naegall, H. (1902). Ber. dtsch. chem. Ges. 35, 3530.

Kitasato, T. (1929). Biochem. Z. 207, 217.

Kline, G. M. & Acree, S. F. (1930). J. Res. nat. Bur. Stand. 5, 1063.

Küchlin, A. Th. (1932). Rec. Trav. chim. Pays-Bas, 51, 887.

Küchlin, A. Th. (1933). Biochem. Z. 261, 411.

v. Lebedev, I. A. A. (1910). Biochem. Z. 28, 1.

Levene, P. A. & Meyer, G. M. (1915). J. biol. Chem. 22, 337.

Levene, P. A. & Mori, T. (1929). J. biol. Chem. 83, 803.

Lewis, W. L. (1909). Amer. chem. J. 42, 301.

Mandl, I. (1950). Arch. Biochem. 25, 109.

Mandl, I. & Neuberg, C. (1952). Arch. Biochem. Biophys. 35, 326.

Mapson, L. W. & Partridge, S. M. (1949). Nature, Lond., 164, 479.

Martos, M. B. & Körösy, F. (1950). Nature, Lond., 165, 369.

Maurer, K. (1929). Ber. dtsch. chem. Ges. 62, 332.

Maurer, K. (1930). Ber. dtsch. chem Ges. 63, 25.

Maurer, K. & Böhme, R. (1936). Ber. dtsch. chem. Ges. 69, 1399.

Maurer, K. & Mahn, H. (1927). Ber. dtsch. chem. Ges. 60, 1316.

Maurer, K. & Müller, A. (1930). Ber. dtsch. chem. Ges. 63, 2069.

Maurer, K. & Petsch, W. (1931). Ber. dtsch. chem. Ges. 64, 2011.

Maurer, K. & Petsch, W. (1933). Ber. dtsch. chem. Ges. 66, 995.

Maurer, K. & Plötner, K. (1931). Ber. dtsch. Ges. 64, 281.

Maurer, K. & Schiedt, B. (1933). Ber. dtsch. chem. Ges. 66, 1054.

Mayer, P. (1911). Biochem. Z. 32, 1.

Mayer, P. (1912). Biochem. Z. 40, 455.

Meunier, J. (1891). Ann. Chim. (Phys.), 22, 415.

Meyer, R. & Reichstein, T. (1946). Helv. chim. acta, 29, 144.

Micheel, F., Kraft, K. & Lohmann, W. (1934). Hoppe-Seyl. Z. 225, 13.

Militzer, W. E. (1944). J. biol. Chem. 154, 325.

Mitchell, I. L. S. & Bayne, S. (1952). Biochem. J. 50, xxvii.

Mohler, H. & Lohr, H. (1938). Helv. chim. acta, 21, 485.

Morrell, R. S. & Bellars, A. E. (1905). J. chem. Soc. 87, 280.

Morrell, R. S. & Crofts, J. M. (1899). J. chem. Soc. 75, 786.

Morrell, R. S. & Crofts, J. M. (1900). J. chem. Soc. 77, 1219.

Morrell, R. S. & Crofts, J. M. (1902). J. chem. Soc. 81, 666.

Morrell, R. S. & Crofts, J. M. (1903a). Proc. Camb. phil. Soc. 12, 115.

Morrell, R. S. & Crofts, J. M. (1903b). J. chem Soc. 83, 124.

Müller, H. & Pechmann, H. (1889). Ber. dtsch. chem. Ges. 22, 2127.

Müller, H. & Reichstein, T. (1938). Helv. chim. acta, 21, 273.

Myrbäck, K. (1938). Svensk kem. Tidskr. 50, 72.

Myrbäck, K. (1939). Svensk kem. Tidskr. 51, 225.

Myrbäck, K. (1940). Svensk kem. Tidskr. 52, 293.

Myrbäck, K. (1941). Svensk kem. Tidskr. 53, 461.

Nef, J. V. (1907a). Liebigs Ann. 357, 259.

Nef, J. V. (1907b) Liebigs Ann. 357, 294.

Nef, J. V. (1910). Liebigs Ann. 376, 1.

Nef, J. V. (1914). Liebigs Ann. 403, 204.

Neher, H. T. & Lewis, W. L. (1931). J. Amer. chem. Soc. 53, 4411.

Nelson, N. (1944). J. biol. Chem. 153, 375.

Neuberg, C. (1902). Z. Ver. Rubenzucker Ind. p. 237.

Neuberg, C. & Kitasato, T. (1927). Biochem. Z. 183, 485.

Neuberg, C. & Strauss, E. (1946). Arch. Biochem. 11, 457.

Niederhoff, P. (1929). Hoppe-Seyl. Z. 181, 83.

Ohle, H. (1931). Die Chemie der Monosaccharide und der Glykolyse. Munich: J. F. Bergmann.

Ohle, H. (1934). Ber. dtsch. chem. Ges. 67, 155.

Ohle, H. & Berend, G. (1927). Ber. dtsch. chem. Ges. 60, 1159.

Ohle, H. & Blell, I. (1931). Liebigs Ann. 492, 1.

Ohle, H. & Hecht, O. (1930). Liebigs Ann 481, 233.

Ohle, H., Henseke, G. & Czyzewski, A. (1953). Ber. dtsch. chem. Ges. 86, 316.

Ohle, H. & Hielscher, M. (1941). Ber. dtsch. chem. Ges. 74, 18.

Ohle, H. & Koller, I. (1924). Ber. dtsch. chem. Ges. 57, 1566.

Ohle, H. & Wolter, R. (1930). Ber. dtsch chem. Ges. 63, 843.

Oldham, J. W. H. & Honeyman, J. (1946). J. chem. Soc. p. 986.

Oppenhauer, R. V. & Oberrauch, H. (1949). An. Asoc. quim. argent. 37, 246.

Paal, C. & Hornstein, F. (1906). Ber. dtsch. chem. Ges. 39, 1361.

Paal, C. & Kinscher, M. (1911). Ber. dtsch. chem. Ges 44, 3543.

Paal, C. & Wiedenkoff, E. (1906). Ber. dtsch. chem. Ges. 39, 2827.

Paal, C.& Zahn, K. (1907). Ber. dtsch. chem. Ges. 40, 1819.

Pacsu, E. & Miller, (1948). J. Amer. chem. Soc. 70, 523.

Pacsu, E., Wilson, E. J. & Graf, L. (1939). J. Amer. chem. Soc. 61, 2675.

Partridge, S. M. (1948). Biochem. J. 42, 238.

Payne, J. H. & Foster, L. (1945). J. Amer. chem. Soc. 67, 1654.

Percival, E. E. & Percival, E. G. V. (1935). J. chem. Soc. p. 1398.

Percival, E. E. & Percival, E. G. V. (1937). J. chem. Soc. p. 1320.

Percival, E. G. V. (1936). J. chem. Soc. p. 1770.

Percival, E. G. V. (1948). Advanc. Carbohyd. Chem. 3, 23.

Petuely, F. (1952). Mh. Chem. 83, 765.

Pigmann, W. W. & Goepp, R. M. (1948). Chemistry of the Carbohydrates, p. 318. New York: Academic Press.

Posternak, T. (1941). Helv. chim. acta, 24, 1045.

Pummerer, R., Cuyot, O. & Birkofer, L. (1935). Ber. dtsch. chem. Ges. 68, 480.

Purdie, T. & Irvine, J. C. (1903). J. chem. Soc. 83, 1021.

Raymond, A. L. (1938). Organic Chemistry, Vol. II, p. 1512. New York: John Wiley and Sons.

Regna, P. P. & Caldwell, B. P. (1944). J. Amer. chem. Soc. 66, 246.

Reichstein, T. & Demole, V. (1936). Festschrift für E. C. Barell, Basel, p. 107.

Reichstein, T., Grüssner, A., & Oppenhauer, R. (1933a). Helv. chim. acta, 16, 561.

Reichstein, T., Grüssner, A. & Oppenauer, R. (1933b). Helv. chim. acta, 16, 1019.

Reichstein, T., Grüssner, A. & Oppenauer, R. (1934). Helv. chim. acta, 17, 510.

Reichstein, T. & Neracher, O. (1935). Helv. chim. acta, 18, 892.

Reichstein, T., Schwartz, L. & Grüssner, A. (1935). Helv. chim. acta, 18, 353.

Richtmyer, N. K., Carr, C. J. & Hudson, C. S. (1943). J. Amer. chem. Soc. 65, 1477.

Richtmyer, N.K., Hann, R. M. &. Hudson, C. S. (1939a). J. Amer. chem. Soc. 61, 340.

Richtmyer, N. K., Hann, R. M. & Hudson, C. S. (1939b). J. Amer. chem. Soc. 61, 343.

Riiber, C. N. & Minsaas, J. (1926). Ber. dtsch. chem. Ges. 59, 2266.

Riley, H. L., Morley, J. F. & Friend, N. A. C. (1932). J. chem. Soc. p. 1875.

Romijn, G. (1897). Z. anal. Chem. 36, 349.

Roseman, S., Abeles, R. H. & Dorrfman, A. (1952). Arch. Biochem. Biophys. 36, 232.

Ruff, O. (1898). Ber. dtsch. chem. Ges. 31, 1573.

Ruzicka, W. (1941). Z. Zuckerind. Bohmen-Mahren, 64, 219.

Salmon, M. R. & Powell, G. (1939). J. Amer. Chem. Soc. 61, 3507.

Salomon, L. L., Burns, J. J. & King, C. G. (1952). J. Amer. chem. Soc. 74, 5161.

Scheibler, C. (1880). Ber. dtsch. chem. Ges. 13, 2212.

Shinoda, J., Sato, S. & Sato, D. (1932). Ber. dtsch. chem. Ges. 65, 1219.

Sjollema, B. & Seekles, L. (1926). Biochem. Z. 176, 431.

Skinner, L. G. (1948). Honours thesis, University of St. Andrews.

Smith, F. (1946). Advanc. Carbohyd. Chem. 2, 79.

Smith, G. F. & Lowry, T. M. (1928). J. chem. Soc. p. 666.

Somogyi, M. (1945). J. biol. Chem. 160, 61.

Sowden, J. C. (1947). J. Amer. chem. Soc. 69, 1047.

Sowden, J. C. (1949). Science, 109, 229.

Sowden, J. C. & Fischer, H. O. L. (1947). J. Amer. chem. Soc. 69, 1963.

Sowden, J. C. & Kuenne, D. J. (1953). J. Amer. chem. Soc. 75, 2788.

Spengler, O. & Pfannenstiel, A. (1933). Z. dtsch. Zuckerind. 85, 546.

Spengler, O. & Pfannenstiel, A. (1935). Z. Wirtschaftsgr. Zuckerind. 85, 547.

Spoehr, H. A. (1910). Amer. chem. J. 43, 227.

Stacey, M. & Turton, L. (1946). J. chem. Soc. p. 661.

Steiger, M. (1935). Helv. chim. acta, 18, 1252.

Stone, I. (1940). U.S. Pat. 2,206, 374.

Sullivan, W. R. (1945). J. Amer. chem. Soc. 67, 837.

Teunissen, H. P. (1930). Rec. Trav. chim. Pays-Bas, 50, 1.

Thannhauser & Jenke, M. (1926). Arch. exp. Path. Pharmak. 110, 300.

Tiffeneau, M. (1928). Org. Synth. 8, 30.

Trevelyan, W. E., Procter, D. S. & Harris, S. S. (1950). Nature, Lond., 166, 443.

Vargha, L. (1934). Ber. dtsch. chem. Ges. 67, 1223.

Vogel, H. (1937). Ber. dtsch. chem. Ges. 70, 1193.

Wadman, W. H. (1952). J. chem. Soc. p. 3051.

Walker, T. K. (1932). Nature, Lond., 130, 582.

Watanabe, K. (1937). J. Soc. trop. Agric., Taihoku Imp. Univ., 8, 381.

Waters, W. A. (1945). Rep. Progr. Chem. 42, 145.

Weidenhagen, R. (1937). Z. Wirtschaftsgr. Zuckerind. 87, 711.

Weygand, F. (1940). Ber. dtsch. chem. Ges. 73, 1296.

Weygand, F. & Schaefer, G. (1951). Chem. Ber. 84, 603.

Willstätter, R. & Schudel, G. (1918). Ber. dtsch. chem. Ges. 51, 780.

Wolfrom, M. L., Konigsberg, M. & Soltzberg, S. (1936). J. Amer. chem. Soc. 58, 490.

Wolfrom, L. M., Schuetz, R. D. & Cavalieri, L. F. (1948). J. Amer. chem. Soc. 70., 514.

Wolfrom, M. L., Schuetz, R. D. & Cavalieri, L. F. (1949). J. Amer. chem. Soc. 71, 3518.

Wolfrom, M. L., Wallace, E. G. & Metcalf, E. A. (1942). J. Amer. chem. Soc. 64, 265.

Woodin, A. M. (1952). Biochem. J. 51, 319.

Young, E. G. & Rice, F. A. H. (1946). J. biol. Chem. 164, 35.

Zerban, F. W. & Sattler, L. (1950). J. Amer. chem. Soc. 72, 3814.

Zerner, E. & Waltuch, R. (1914). Mh. Chem. 35, 1025.

Zervas, L. (1930). Ber dtsch. chem. Ges. 63, 1689.

Additional references:

Bernhauer, K. & Gorlich, B. (1935). Biochem. Z. 280, 367.

Bernhauer, K. & Irrgang, K. (1935). Biochem. Z. 280, 360.

Harrison, K. (1931). Biochem. J. 25, 1016.

Kluyver, A. J. & de Leeuw, R. (1924). Tijdschr. u. Vergelijk. Geneesk. 10, Afl. 2-3.

Lockwood, L. B., Tabenkin, B. & Ward, G. E. (1941). J. Bact. 42, 51.

Muller, D. (1931). Biochem. Z. 232, 423.

Stubbs, J. J., Lockwood, L. B., Roe, E. T., Tabenkin, B. & Ward, G. E. (1940). Ind. Engng Chem. 32, 1626.